JOURNAL

OF THE

AMERICAN SOCIETY

OF

SUGAR BEET TECHNOLOGISTS

VOL. X, NO. 6 JULY 1959

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JOURNAL
of the
American Society of Sugar Beet Technologists

Volume X
Number 6
July 1959

Published quarterly by

The American Society of Sugar Beet Technologists
Office of the Secretary
P. O. Box 538
Fort Collins, Colorado, U. S. A.

Subscription prices:

$4.50 per year, domestic
$5.00 per year, foreign
$1.25 per copy, domestic
$1.40 per copy, foreign

Made in the United States of America

TABLE OF CONTENTS

	Author	Page
Yield comparisons from chemically and hand-weeded sugar beets under watergrass conditions in California	*L. M. Burtch* *C. M. Carlson*	467
Rapid determination of moisture in pressed sugar beet pulp	*Alan E. Goodban* *T. Jaouni* *R. M. McCready*	478
Fffects of storage on raffinose content of sugar beets I. Varietal changes occuring during storage	*P. E. Finkner* *M. D. Finkner* *R. F. Olson*	481
Effects of storage on raffinose content of sugar beets. II. Evaluation of variance components for optimum sample size determination	*M. D. Finkner* *R. E. Finkner* *R. F. Olson*	489
Response of sugar beets to nitrogen depletion in relation to root size	*R. S. Loomis* *Albert Ulrich*	499
Influence of the seedball on speed of germination of sugar beet seeds	*F. W. Snyder*	513
The use of systemics di-syston and thimet to control virus yellows in California	*J. C. Hoff* *R. H. Heath* *D. D. Dickenson*	521
Some chemical and physiological characteristics of inbred lines of sugar beets	*George K. Ryser* *Myron Stout* *Albert Ulrich* *F. V. Owen*	525
Distribution of cyst- and gall-forming nematodes of sugar beets in the United States	*Fields E. Caveness*	544
Some effects of gibberellin on stem elongation and flowering in sugar beets	*F. W. Snyder* *S. H. Wittwer*	553

Yield Comparisons from Chemically and Hand-Weeded Sugar Beets Under Several Watergrass Conditions in California

L. M. BURTCH AND C. M. CARLSON[1]

Received for publication April 8, 1958

Watergrass (*Echinochloa crusgalli*), an annual grass which does not germinate at low temperatures and is killed by frost, is a common weed in the Central Valley of California from May to October. Since, in watergrass areas, most sugar beets are planted in January, February, and March, watergrass does not usually become a problem until after beets are thinned and off to a good start. Watergrass in these fields thus becomes serious in summer after beet foliage development prohibits efficient machine cultivation.

In recent years, a number of trials have been conducted in California in an attempt to control annual grasses with chemicals — especially watergrass in established sugar beets. One material, sodium 2, 2 dichloropropionic acid (dalapon), has offered considerable promise in that it has reportedly been applied directly to beets at rates which will kill grasses without showing serious injury to beets (1) (3)[2]. Although sugar beets are tolerant to dalapon, yield reductions have occurred where the material has been applied directly to sugar beet foliage, especially when the material has been used at temperatures above 70° F. (2). The adverse influence of dalapon on yields suggests directed or shielded sprays be used under California watergrass conditions. Southwick *et al.* (2) report a cooperative experiment with Loomis at the University of California, Davis, showing less toxicity with directed sprays than with over-all sprays applied to beets two months after thinning.

The reported effectiveness of dalapon in controlling annual grasses in sugar beets without excessive damage to the crop suggested possible application to the watergrass problem in sugar beets under California's Central Valley conditions.

Since 1955, a series of experiments have been conducted with the following objectives:

(1) To determine the quantitative effect of watergrass competition on sugar beet yields under actual field conditions.
(2) To improve sugar beet yields by hand hoeing and by use of dalapon both by directed and over-all sprays.
(3) To compare the effectiveness of single and repeated applications on the above treatments.

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[2] Numbers in parentheses refer to literature cited.

(4) To test sugar beet varieties for possible tolerance to dalapon.

Methods and Materials

Four experiments were conducted in order to compare grass control effectiveness of 5 or 6 treatments under a wide range of watergrass conditions. A randomized block design with 5 or 6 replications was used with individual plots consisting of 4 (single or double row) beds 60 or 80 feet long. Yields were obtained by hand harvesting the center 2 beds of each plot. A small trailer-mounted sprayer was developed to apply both directed and over-all applications of chemical.

Hoeing as required for complete control, and an uncontrolled grass check were included in all comparisons. Spray treatments were applied at least once when beet foliage was 8 to 16 inches high and most of the watergrass about 3 to 6 inches in height but with sizes ranging from newly emerged seedlings to small amounts in the heading stage. All applications were made in midsummer with temperatures ranging from 80 to 95° F. Dalapon (85 percent sodium salt) was applied in thirty to fifty gallons of water per acre at 30 p.s.i. gage. Ground speed varied from ½ to 1½ m.p.m. and fan type Teejet nozzles (80° angle), numbers 8001 and 8002 were used to obtain desired coverage and rates. In the case of broadcast applications, nozzles were arranged on the boom for full coverage. For directed applications, shields were designed to permit full ground coverage around the crown of the beet and in the row center as well as the entire area between rows. In actual field practice some of the older, lower leaves of beets received some spray.

Observations and Results

Severe Watergrass Infestation

The first experiment was established on beets expected to be severely infested with watergrass although the field was relatively weed free when selected for the experiment. On May 28, 1957, the plots designated for hand weed control were hoed for the first time.

By mid-June, newly emerged watergrass was thick in the irrigation furrows and on the bed centers. Grass in the beet rows (untouched by cultivation) was now about six inches high. The grass in the hoed plots was again removed on June 15.

The dalapon treatments were applied on July 11 at five pounds per acre. At this time the grass ranged in size from newly emerged seedlings to grass in full bloom with most grass ranging from four to six inches high and beet foliage about 14 inches high. Grass was growing actively with adequate moisture and

temperatures were about 90° F. when the material was applied. Coverage and wetting of the grass appeared to be excellent, but the application might have been more effective had it been possible to have applied the material a week earlier.

Dalapon plots could easily be distinguished by dry appearance and purplish color of grass by the end of July. The hoeing treatment, while outstanding by comparison with all other treatments, showed solid grass coverage nearly as high as the foliage. The third and final hoeing was completed on August 20. Observations in late August showed the dalapon holding older grass in fair control with the directed application showing only slightly less control than the over-all application, however, newly emerged grass was apparent in all dalapon treated plots.

The plot was harvested on October 15. The treatments used, cost, degree of weed control, yield, sucrose content, sugar per acre, and stand of the plot are shown in Table 1. It is apparent that uncontrolled watergrass decreased yields from 28.4 to 16.3 tons per acre. Control of watergrass by three hoeings was good but cost approximately $90.00 an acre. Control of watergrass by dalapon with shields was about equal to the over-all application but neither treatment was significantly better in yield than the check. The July 11 application controlled most of the grass present at the time of application, except for large grass in bloom, but provided no control over grass emerging after treatment.

Table 1.—Sugar Beet Yields Under Severe Watergrass Conditions as Influenced by Hand-Hoeing, Cultivation, and Post-Emergence Chemical. Beets Were Planted in February, Near Firebaugh, California, and Dalapon Was Applied on July 11[1].

Treatment	Rate	Approx. Cost	% Grass Control at Harvest[3]	Gross Sugar Tons/Acre	Beets Tons/Acre	Sucrose %	Beet Stand at Harvest (100′ Row)
Hoeing[2]	3 Times	$90	88	4.28	28.4	15.1	156
Dalapon (Directed)	5 Lbs.	$ 9	42	2.64	17.9	14.7	128
Dalapon (Over)	5 Lbs.	$ 7	44	2.53	17.4	14.6	131
Cultivation	2 Times	$ 4	25	2.48	16.5	15.0	145
None			20	2.40	16.3	14.6	123
Mean				2.87	19.3	14.8	137
LSD P = .05				0.54	3.3	NS	
LSD P = .01				0.74	4.5	NS	

[1] Beet foliage varied from 10 to 14" in height and the majority of watergrass was 5 inches in height when treated. Some leaf burn was observed on dalapon treated beets.

[2] May 28, June 15, August 20.

[3] Percent grass control is based on quantitative visual percentage estimate with 0% indicating solid grass coverage and no beets visible through the grass.

Moderate Watergrass Infestation

Another experiment was established July 19, 1957, on beets planted in late March and thinned in late May. Beet foliage was approximately 15 inches high with an intermediate infestation of watergrass present. Watergrass ranged in size from 2 inches to 10 inches, with about 10 percent beginning to bloom. Most of the grass present was in the beet row (30" single rows) and on the bed shoulders with only small amounts present in the furrows.

Hoeing was completed on July 20. Three dalapon treatments were established on July 19, one over-all rate at 3.2 pounds per acre, a directed rate at 3.2 pounds, and a heavier directed rate of 4.7 pounds per acre.

Observations taken on August 1 showed considerable tall grass (30 inches high) in the checks and very little response to dalapon. By August 15, however, some leaf burn was noticed on the over-all application and grass stunting and die-back was noticeable, even on the lower dalapon rates. By harvest, most grass was nearly 5 feet high and the infestation was more severe than had been expected from notes taken in mid-July. Checks showed heavy grass concentrations between beets in the row and on the shoulders of the beds with some grass in the furrows. Plots treated with dalapon showed nearly as much grass as the check treatments. The vigor of the grass was reduced by all applications, however. The results of this test are shown in Table 2.

Table 2.—Sugar Beet Yields Under Moderate Watergrass Conditions as Influenced by Hand-Hoeing, Directed and Over-Foliage Sprays of Dalapon. Beets Were Planted in Late March Near Yuba City, California, and Hoeing and Chemical Treatments[1] Were Applied on July 19-20, 1957.

Treatment	Rate	Approx. Cost	% Grass Control at Harvest[2]	Gross Sugar Tons/Acre	Beets Tons/Acre	Sucrose %	Beet Stand at Harvest (100' Row)
Hoeing	1 Time	$12	90	3.92	26.2	15.2	170
Dalapon (Directed)	3.5 Lbs.	$ 7	50	3.24	21.6	15.1	152
Dalapon (Over)	3.5 Lbs.	$ 5	50	3.22	21.8	14.8	184
Dalapon (Directed)	4.7 Lbs.	$ 9	50	3.03	19.8	15.3	140
None			40	3.01	19.9	15.2	140
Mean				3.28	21.9	15.1	157
LSD P = .05				0.62	4.6	NS	
LSD P = .01				NS	NS	NS	

[1] Beet foliage averaged 15" in height and watergrass ranged from newly emerged to near maturity when treated. Leaf burn was observed on dalapon treatments.

[2] Percent grass control is based on quantitative visual percentage estimate with 0% indicating solid grass coverage with no beets visible through the grass.

One hoeing at an estimated cost of $12.00 provided excellent grass control for the season and significantly increased tonnage over all other treatments. Dalapon treated plots showed some yield increase over the check but this increase was not significant at the 5% level of probability.

Sparse Watergrass Infestation

Another experiment was established on a field planted May 1 and thinned June 15, 1957. The field was selected as being typical of a light watergrass infestation on a late planting. The furrows were clean and the grass was small, three to six inches high on beets having foliage 10 inches high.

The soil was fairly dry, but both beets and grass showed good color and appeared to be growing actively. One over-all and two directed applications of dalapon were made on July 24. Rates were increased to 6.5 lbs./acre on this experiment to attempt a wider range of control, and one high rate (9.6 lbs./acre) was included to determine if some pre-emergence grass control might be obtained in the furrows later in the season. Directed applications overlapped around the crown as before, but a larger percentage of the lower leaves received material because of the small beet size. The hoeing treatment was completed at an estimated cost of $7.00 per acre.

By August 8, leaf burn and stunting was evident on the over-all treated plots and on the outer leaves of the beets receiving directed applications. The center leaves of the shielded beets were free from injury symptoms except for some burn present on the center leaves at the highest rate of dalapon. By late August, all sprayed treatments were clearly defined. Grass was severely stunted and dying, the hoed plots were clean, but grass competition in the check plots was less than had been expected. Late emerging watergrass did not develop as expected and the experimental area remained much the same until harvest in November with only scattered grass showing above the foliage. Only the check area showed much tall watergrass and it was concentrated mainly in the beet row.

Harvest results obtained on November 6, showed that the grass problem was not severe for any treatment (Table 3). A single hoeing again produced the cleanest beets having the highest yield. Yields were reduced in the dalapon treatments largely from beet injury; however, the treatments were satisfactory in controlling watergrass. The directed treatments showed effective grass control with lower yield reductions than the over-all treatment although none of the differences reached statistical significance at the 5% level.

Table 3.—Sugar Beet Yields Under Sparse Watergrass Conditions as Influenced by Hand-Hoeing, Directed and Over-Foliage Sprays of Dalapon. Beets Were Planted Near Tracy, California, in May and Hoeing and Chemical Treatments[1] Were Applied on July 25-26, 1957.

Treatment	Rate	Approx. Cost	% Grass Control at Harvest[2]	Gross Sugar Tons/Acre	Beets Tons/Acre	Sucrose %	Beet Stand at Harvest (100' Row)
Hoeing	1 Time	$ 7	95	3.01	19.3	15.6	180
None			75	2.72	17.4	15.6	164
Dalapon (Directed)	9.5 Lbs.	$15	85	2.64	17.5	15.1	178
Dalapon (Directed)	6.5 Lbs.	$11	85	2.54	16.7	15.1	168
Dalapon (Over)	6.5 Lbs.	$ 9	80	2.37	15.4	15.5	174
Mean				2.66	17.3	15.4	173
LSD P = .05				NS	NS	NS	

[1] Beet foliage averaged 10" in height and watergrass ranged between 3 and 6" in height. Leaf burn was observed on dalapon treatments.

[2] Percent grass control was based on quantitative visual percentage estimate with 0% indicating solid grass coverage with no beets visible through the grass.

The experiments conducted in 1957 indicated clearly that one application of dalapon was not sufficient for seasonal control of watergrass under severe conditions. Single and repeat applications of the hoe should be compared with directed sprays of dalapon in order to obtain more economical seasonal control of watergrass.

Single and Repeat Applications on Severe Watergrass Infestations

An experiment, designed to make these comparisons, was established July 22, 1958, on beets planted in late May and thinned about July 1. Beet foliage was approximately eight inches high with a severe watergrass problem present. Watergrass ranged in size from one to five inches. The experiment was replicated six times in a randomized block design. The hoeing treatments were completed on July 22 at an estimated cost of $45 per acre. Three dalapon treatments were also established on July 22 at rates of 4.7 pounds of dalapon per acre. One treatment was applied over the foliage of the beet, but the remaining treatments were directed as in the 1957 experiments.

Heavy infestations of grass appeared in all plots in early August. On August 19 all beets designated for extra hoeing were hoed at an estimated cost of $33 per acre. On August 20, directed sprays of dalapon were applied to all beets previously sprayed over the foliage and to one set of treatments that had received early directed sprays.

Observations taken after the July treatments and prior to the August treatments showed leaf burn on all dalapon sprayed beets with watergrass showing stunted new growth and a gray-green color. Hoeing treatments were outstanding and the check beets were completely hidden by watergrass. New grass growth was apparent in all plots. By mid September, beets treated twice, were improved over those receiving single applications. All beets hoed twice were outstanding, however, and beets hoed once were nearly as vigorous. Beets in check plots were extremely stunted and completely hidden by tall watergrass.

Harvest results obtained on November 11 are shown in Table 4. The extreme reduction in yields resulting from watergrass competition is apparent from the data. Satisfactory yields were obtained only where watergrass was effectively controlled by hand hoeing. Dalapon treatment, in the other experiments, failed to improve yields sufficiently to be practical.

Variety Tolerance

Possible varietal tolerance to dalapon in the absence of watergrass was tested in 1955 when five pounds of dalapon was applied at two stages of beet growth to six sugar beet varieties differing

Table 4.—Sugar Beet Yields Under Severe Watergrass Conditions as Influenced by Hand-Hoeing, Directed and Over-Foliage Sprays of Dalapon. Watergrass Condition Was Severe. Beets Were Planted Near Davis, California, in Late May and Treatments[1] Were Started on July 22, 1958.

Treatment	No. Applications	Approx. Cost	% Grass Control at Harvest[2]	Gross Sugar Tons/Acre	Beets Tons/Acre	Sucrose %	Beet Stand at Harvest (100′ Row)
Hoeing	2	$78	95	2.83	17.6	16.1	90
Hoeing	1	$45	75	2.28	14.3	15.9	75
Dalapon (Overall + Directed)	2	$15	55	1.10	7.0	15.5	65
Dalapon (Directed)	1	$ 7	30	1.06	6.9	15.4	55
Dalapon (Directed)	2	$16	20	0.91	5.9	15.4	55
No Control			5	0.49	3.2	15.3	40
*Rate consisted of 4.7 lbs. per acre of dalapon each application.							
Mean			47	1.44	9.2	15.6	63
LSD P = .05				0.35	2.3	0.4	
LSD P = .01				0.47	3.1	0.5	

[1] Beet foliage averaged 8" in height and watergrass ranged between 1 and 5 inches in height. Leaf burn was observed on all dalapon treatments.

[2] Percent grass control was based on quantitative visual percentage estimate with 0% indicating solid grass coverage with no beets visible through the grass.

widely in genetic background. The experiment was planted on May 24, and harvested November 15, 1955. Varieties tested were US 400, S-1 (a selection from US 15), US 75, US 56 and two experimental varieties. Untreated checks of each variety were replicated six times in a randomized block design. Applications of dalapon were made in late June, shortly after thinning, and in early August.

Table 5.—The Influence of Foliar Applications of Dalapon at 5 Pounds per Acre on Six Varieties of Sugar Beets Under Weed-Free Conditions. Values Given are Averages of Six Replications. Dalapon Was Applied at Two Stages of Growth, Shortly After Thinning and Six Weeks Later.

Treatment	Application Date	S-1	US 400	US 75	US 56/2	A5327	A54103	Mean
Sugar per Acre—Expressed as Percent of Check								
None (Check)		100	100	100	100	100	100	100
Dalapon	6/25	89	87	80	84	82	89	85
Dalapon	8/3	66	66	73	74	65	78	70
LSD between varieties at P = .05 (Var)		13						
LSD P = .01 (Var)		17						
Beets per Acre—Expressed as Percent of Check								
None (Check)		100	100	100	100	100	100	100
Dalapon	6/25	93	87	84	91	87	92	89
Dalapon	8/3	74	76	80	80	76	83	78
LSD between varieties at P = .05 (Var)		10						
LSD P = .01 (Var)		13						
Sucrose Percent—Expressed as Percent of Check								
None (Check)		100	100	100	100	100	100	100
Dalapon	6/25	95	100	95	100	95	95	96
Dalapon	8/3	92	86	90	92	92	93	90
LSD between varieties at P = .05 (Var)		3						
LSD P = .01 (Var)		5						

The results of this experiment are shown in Table 5. The statistical interaction of variety times treatment did not reach the five percent level of significance for sugar per acre, tonnage, or sucrose percentage indicating that all varieties reacted similarly to the application of dalapon. The results indicate that dalapon reduced yields for all varieties and that late applications produced more serious yield reductions than the early application.

It has been demonstrated repeatedly under California conditions that the presence of an excess of nitrogen at harvest has a depressing influence on the sucrose concentration in sugar beets. Since the late application of dalapon reduced top growth and root development, it is possible that the reduction in sucrose concentration was the result of unused nitrogen at harvest, rather than a direct influence of dalapon.

Discussion

Watergrass has long been recognized as the most serious weed problem in the Central Valley area of California. Because watergrass generally becomes most severe in midsummer and fall after the last cultivation, post-emergence chemical control methods seem most desirable. This approach permits dealing with the problem as it arises, but also places severe restrictions on the material to be used. Dalapon almost conforms to these limitations in that it kills watergrass and volunteer grain at rates which will not kill sugar beets. Unfortunately, dalapon, even at low rates, has retarded beet growth under California conditions, and this has been reflected in reduced yields at harvest. This is particularly true when dalapon is used as a foliar application, but directed or shielded application should reduce this toxicity.

In all tests reported, dalapon provided some control over watergrass under all conditions tested, but the control was not as effective as needed, and yields were not satisfactorily improved by its use. The reasons for this apparent lack of control have been two-fold. 1. sensitivity of beets to dalapon and 2. the continued appearance of new grass throughout the summer. Since it would be most desirable to use dalapon only once during the season, a small portion of early emerging grass was approaching maturity (generally in the beet row) in most of the experiments reported by the time the main infestation reached the stage for most effective control. After treatment, more grass usually emerged with successive irrigations. Thus, the time for most efficient chemical application is very important and it will be difficult to find one period during which a single foliar application of post-emergence chemical will control all grass present and also prevent future germination of watergrass seeds.

This same objection holds true for hoeing except that hoeing will generally control grass over a wider size range than chemicals, and also corrects the unsightly appearance of the larger watergrass. Furthermore, hoeing destroys some of the newly emerged grass that is too small to be destroyed by absorbed chemicals. Dalapon, for example, is absorbed largely through the leaves, action is slow and newly emerged grasses have such

a small leaf area that they often escape contact and absorption of the material.

Chemical application with dalapon could not be considered satisfactory in controlling watergrass in any of the tests reported. Under severe watergrass conditions, neither one nor two chemical applications were sufficient to provide seasonal weed control. The data indicate, however, that watergrass control is highly desirable from an economic standpoint and in two of four tests, $7.00 to $15.00 in hand labor adequately controlled the grass problem for the season. In the severe infestations (Tables 1 and 4) the grass infestation was so heavy that the fields would have been better not planted to a row crop.

The second 1957 experiment which was carried out under moderate watergrass infestation might well have been improved had the application of dalapon been earlier and at higher rates (Table 2).

Repeated observations of fields with a serious watergrass problem show that obtaining and holding a good stand of beets down the row is one of the best methods of discouraging watergrass growth in summer and that late cultivations with side knives can save many dollars in hoeing and in reducing harvest costs. Further work with directed sprays with emphasis on materials, timing, and frequency of application could be of value because hand labor in sugar beet fields is becoming scarce as well as expensive. As long as hand labor is available, however, judicious use of the hoe may often pay dividends in increased yields and clean fields. (Tables 1, 2, 4.)

Summary and Conclusions

Several experiments testing dalapon for control of watergrass in sugar beets and the resulting influence of the material on sugar beet yields are reported. Comparisons included timing, frequency of application, directed and over-all sprays, and several rates of material. Hand hoeing checks were included together with checks in which the watergrass was not controlled (field practice).

All dalapon applications were made in mid-season when beet foliage was 8 to 15 inches high with watergrass ranging from newly emerged to 12 inches high with most about 5 inches in height.

Dalapon was found to provide some, but insufficient, control over watergrass under all situations and yields were not satisfactorily increased by its use.

Uncontrolled watergrass reduced yields from 2 to nearly 14 tons per acre depending on severity of grass competition as compared to hand hoed plots that were kept relatively clean.

Hand hoeing produced the best economic control of watergrass and resulted in the highest yields in all cases.

Studies on differential response to dalapon by different sugar beet varieties showed no significant variety times treatment interaction, indicating that none of the varieties tested appeared more or less affected by the dalapon. Yields of all varieties were reduced by the use of dalapon.

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Rapid Determination of Moisture in Pressed Sugar Beet Pulp

ALAN E. GOODBAN, T. JAOUNI AND R. M. MCCREADY

Received for publication May 21, 1958

A large proportion of the sugar beet pulp produced in the U.S. is pressed and dried for use as a feed, and it is necessary to determine the moisture content of pressed pulp rather frequently as a control on pressing operations. The usual method of oven drying involves a lag of several hours between sample collection and results. The purpose of this report is to present results on a chemical method for solids determination (1, 2, 3, 4)[2] which may be used as an alternative to oven drying to the rapid method of Cotton et. al. (5).

Since the solids in plant materials are largely organic, it should be possible to determine total solids by measuring the dichromate necessary for complete oxidation and to calculate moisture difference. A rapid reproducible method, easily adaptable to beet pulp, has been developed by Launer and Tomimatsu 1, 2, 3, 4)[2] for the determination of moisture in rice, prunes, corn, potatoes, peas, and pineapple-rice pudding.

Details of the procedure are to be found in published reports 1, 2). An outline of the procedure is as follows: 25.00 ml. of 1.835N potassium dichromate is added to a weighed sample of material, calculated to reduce not more than 80 percent of the dichromate. About 30 ml. of concentrated sulfuric acid is carefully added while the mixture is stirred with a magnetic stirrer. It is important that the first 10 ml. be added slowly and that foaming caused by the rapid initial oxidation be allowed to subside before the remainder of the acid is added. The heat of dilution of the acid raises the temperature of the mixture sufficiently to oxidize the organic material within 4 minutes. The reaction mixture is then diluted with 150 ml. of water and the excess dichromate titrated electrometrically with ferrous ammonium sulfate. A pH meter with platinum-calomel electrodes can be used, or a simple system can be arranged with about 10 dollars worth of equipment (1) to detect the end point. The entire procedure can be completed within 10 minutes after the sample has been weighed. Use of magnetic stirring and avoidance of external heating are essential for reproducible oxidation (3). Care must be taken when adding the sulfuric acid that the

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[1] Western Regional Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Albany 10, California. Presented at 10th Meeting of American Society of Sugar Beet Technologists, Detroit, Mich., Feb. 4-6, 1958.

[2] Numbers in parentheses refer to literature cited.

foam produced during the initial oxidation does not deposit some of the solid sample on the side of the beaker above the final level of the oxidizing solution.

In order to calculate solids in a sample, it is necessary to know the dichromate consumed in oxidizing a given amount of the dry material in the sample. Moisture can then be calculated from the formula:

$$\text{Moisture } \% = 100\ (1 - F \times D)$$

where D is ml. dichromate per gm. of wet sample and F is gm. dry material per ml. dichromate. The factor F can be determined from the formula by comparison with a reference method of moisture determination.

A major point of interest in consideration of this method is the accuracy and precision of the factor F. Listed in Table 1 are the F values for various materials, taken from published reports of Launer and Tomimatsu (1, 2, 3, 4) and for beet pulp, determined here on one sample of beet pulp dried in the oven for comparison. The uniformity of the factor is striking, showing a range of only 11% for the plant materials listed. This can be attributed to the fact that the solids of each of these materials are largely carbohydrate.

Table 1.—*F* Values[1]

Material	*F*	Material	*F*
Rice	0.01247	Glucose	0.01385
Prunes	0.01382	Sucrose	0.01319
Corn	0.01268	Polyhexose	0.01248
Potatoes	0.01338	Peas	0.01361
Pineapple-rice pudding	0.01333	Sugar beet pulp	0.01302
		S.D.	0.00004

[1] Gm. of solids equivalent to 1 ml. of 1.835 N dichromate.

The sugar beet factor given in Table 1 was determined on one sample of dried and ground pulp. The pulp was from beets which had been stored for several months prior to diffusion. The F factor for beet pulp can be more accurately established by using several different batches of pulp. A serious source of error in any procedure (the dichromate as well as oven drying) lies in sampling techniques. Table 2 shows the variations in moisture levels found by different sampling methods. Oven moisture was determined at 60° C., 18 mm. Hg. for 16 hours, and dichromate moisture by use of F = .01302, sample chosen to contain 200 to 250 mg. solids. Four to 6 replicates were run to determine standard deviations.

Table 2.—Variation in Moisture Level With Sampling Method.

Sampling Method	Oven Moisture	S.D.	Dichromate Moisture	S.D.
5-Gm. grab samples	93.4	0.17	——	——
Pulp chopped with knife, 4-gm. samples	93.69%	0.13	93.60%	0.16%
Blended with 2 vol. water, 10-gm. samples	93.82%	0.06	93.60%	0.03%

We would like to call attention to the fact that in a pulp of 90 percent moisture, an error of 1 percent in solids determination results in only 0.1 percent error in moisture content, so that when determinations are made with the dichromate method, the higher the water content of the sample, the more accurate is the moisture determination.

Although only a limited number of trials have been made on sugar beet pulp, it appears that the solids in samples of sugar beet pulp can be determined by the dichromate heat-of-dilution method as precisely as by vacuum oven desiccation. The method requires only about 10 minutes after the sample is weighed, and only 4 minutes per sample for a series of samples. No expensive equipment is required.

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Effects of Storage on Raffinose Content of Sugar Beets I. Varietal Changes Occurring During Storage

R. E. Finkner, M. D. Finkner and R. F. Olson[1]

Raffinose is one of the many unwanted melassigenic substances, commonly called impurities, which interferes in the recovery and refining of sucrose. It is a dextrorotatory, trisaccharide compound with the chemical make up of $C_{18}H_{32}O_{16}.5H_2O$ and has very little commercial value. Like sucrose, it is precipitated by lime in the saccharate process and is mostly retained in the syrups because of continued recycling of the juices in the sugar factory. Zitkowski (9, 10, 11)[2] recognized the troublesome characteristics of raffinose and published methods of isolating it from beet sugar products. He used artificial mixtures of sucrose and raffinose and demonstrated by photomicrographs that small quantities of raffinose could interfere with the size and shape of the sucrose crystal. This is of importance to the beet sugar manufacturer whose aim it is to produce crystals as uniform as possible.

Breeding of sugar beets for low raffinose content was made possible by the development of the paper chromatography method of determination as described by Brown (2) and Serro and Brown (6). With this new technique it was possible to determine the raffinose content of individual beets very rapidly. Brown and Wood (3) also demonstrated that varieties differed in their raffinose content when grown under the same conditions and concluded that these differences were due to the genotype of the varieties. They also reported that the raffinose content of the same variety could vary from location to location, and that some increase in raffinose content was noticed during storage. Finkner and Bauserman (5) reported an approximate 30 percent increase in raffinose content from the same varieties which were harvested three weeks later in a date of harvest test. Progeny performance of roots which were selected from the same population for high and low raffinose content have been reported by Wood (7) and Finkner and Bauserman (5). Both papers concluded that the raffinose content of beets could be significantly increased or decreased by mass selection. Wood et al (8) studied the inheritance of raffinose production in sugar beets and reported the number of effective factor pairs for production of raffinose between the two parents used was about five and at least one is

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[2] Numbers in parentheses refer to literature cited.

non-isodirectional and all are equal in magnitude. In the crosses studied, neither dominance, heterosis, nor linkage appeared to be involved. Quantitatively, the factors for raffinose production in the two parents followed an arithmetic scale and consequently were additive. Correlation coefficients between raffinose and a number of other chemical characteristics of sugar beets have been calculated by several investigators (1) (4) (5). Most of them have reported a negative association of raffinose with sucrose percent, indicating some gain in sucrose should be made as raffinose is reduced by breeding methods. This also would be helpful from the processors standpoint.

The objectives of the present investigation were: To determine if raffinose content increased in topped beets during storage; to ascertain if all varieties would react similarly during storage, regardless of their original raffinose content.

Materials and Methods

The roots used in this storage study were taken from the border rows of the 1955 Chemical tests which have been reported by Finkner and Bauserman (5). The investigation was designed to have four varieties, four replications and four storage periods in a split plot design. Three of the four varieties used were selections from an elite stock of American No. 1. Strain 54-407 was the result of a random selection of mother beets from American No. 1, while 54-410 and 54-411 were selected for high and low raffinose content respectively from the same parental variety. The fourth variety was American No. 2 which is the commercial variety grown in the Rocky Ford, Colorado, area.

The original Chemical Test (5) had eight replications but beets from replications 1 and 2; 3 and 4; 5 and 6; 7 and 8, were bulked together to form the four replications of this storage test. A total of 35 beets of each variety in each of the four replications were topped and stored in the Rocky Ford root cellar on October 24, 1955. Seven beets of each variety within each replication were selected at random and stored together in crates, or a total of 28 beets per crate. The 16 crates, one crate per each replication and for four different storage dates, were randomized and stacked in the root cellar at a temperature of approximately 4 degrees Centigrade. This temperature varied from 2 to 6 degrees throughout the storage periods. The remaining beets, seven roots of each of the four varieties of each replication, were bulked together in large onion sacks and all four sacks, each containing a single replication, were buried in the storage pile at Manzanola, Colorado, on the evening of October 25, 1955.

After three weeks the beet samples in the storage pile were analyzed and compared with samples stored three weeks in the root cellar. Beet samples stored in the root cellar were analyzed

at 3 week intervals. The non-stored check was in storage one day while the experiment was being set up.

Each beet in the root cellar was sampled twice by rasping and two determinations made on each pulp sample, making a total of four readings for every beet in storage. The raffinose content was determined by the paper chromatographic method and is reported as percent on dry substance.

Results and Discussion

On October 24 all beets for the storage test were harvested and kept in the root cellar at Rocky Ford, Colorado. The next day all roots were crated and stored according to the random design, and one set of beets was buried in the beet storage pile at Manzanola, Colorado. The first group was rasped on October 26th and it was considered as the non-treated or non-stored group as it was in the root cellar for only one day.

Three weeks later the group of roots stored in the Manzanola storage pile was taken out of the pile and sampled. During the process of removing these beets the drag-line tore open one sack and all the beets from the first replication were lost. The same day, November 15th, a similar group of roots was removed from the root cellar at Rocky Ford and they also were sampled.

These two groups of beets were analyzed in a split plot design with three replications and the analysis of variance is given in Table 1.

Table 1.—Analysis of Variance of Four Varieties and Two Different Types of Storage.

Source of Variation	D.F.	M.S.	F. Value
Total	671		
Replications	2	.3237	
Varieties	3	1.5624	10.76[1]
Error A (Rep. x Var.)	6	.1450	
Storage	1	.1357	0.41
Variety x Storage	3	.2996	0.91
Error B	8	.3307	
Beets within plots	144	.0650	
Rasping from same beet	168	.0141	
Determinations	336	.0113	

[1] Significant at the one percent level.

No significant differences were detected for raffinose content between the roots stored in a commercial storage pile and the roots stored in the root cellar. Therefore, either place could be used for testing different varieties and consecutive storage periods. This experiment was designed to utilize the root cellar.

The means of the four varieties under both types of storage conditions and the general means of each storage type are shown in Table 2.

Highly significant differences were detected for varieties. This was expected since selection pressure was applied in opposite directions in two of the varieties for raffinose content. It also had been shown previously by progeny tests (5) that selections 54-410 and 54-411 contained significantly different amounts of raffinose.

The two remaining sets of beets in the root cellar were rasped on December 7 and December 28. The four variety means and the storage means are shown in Table 3 and Figure 1. These means are from four replications while the means in Table 2 are from only three replications.

Table 2.—Variety and Storage Means for Raffinose as Percent on Dry Substance.

Varieties	Storages		Variety Means
	Root Cellar	Storage Pile	
American No. 2	.504	.377	.441
54-410	.617	.547	.582
54-407	.389	.426	.408
54-411	.334	.380	.357
Storage Means	.461	.433	

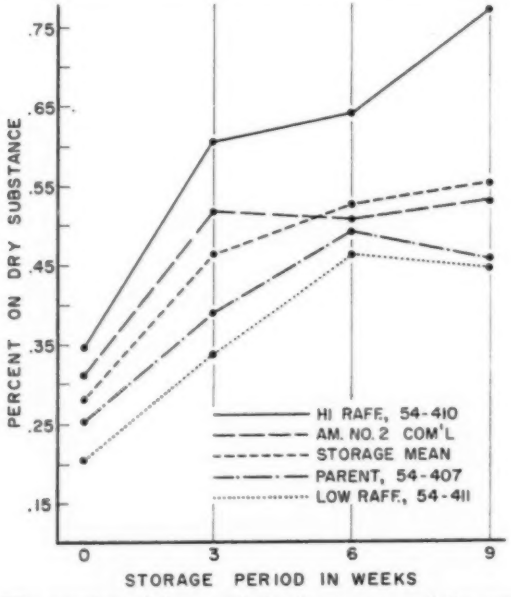

Figure 1.—The reaction of four varieties in storage and the general mean.

Table 3.—Variety and Storage Period Means for Raffinose as Percent on Dry Substance.

Variety	Storage Period in Weeks				Variety Means
	0	3	6	9	
American No. 2	.312	.520	.507	.529	.467
54-410	.350	.608	.643	.773	.594
54-407	.255	.387	.492	.455	.397
54-411	.205	.338	.464	.448	.364
Storage Means	.281	.463	.526	.551	

Each variety mean at each storage period was the result of rasping seven beets from each replication twice, and running two determinations on each pulp sample from each rasping. Therefore a total of 112 raffinose determinations were made to estimate the mean of a single variety for any one storage period. The storage means for each period were estimated by using these 448 raffinose determinations. The analysis of variance of these four varieties and the four storage periods are shown in Table 4.

Table 4.—Analysis of Variance of Four Varieties and Four Storage Periods.

Sources of Variation	D.F.	M.S.	F. Value
Total	1791		
Replications	3	.8649	
Varieties	3	4.6281	16.11[2]
Error A (Rep. x Var.)	9	.2872	
Storage	3	6.7044	61.96[2]
Varieties x Storage	9	.2812	2.60[1]
Error B	36	.1082	
Beets within plots	384	.1156	
Rasping from same beet	448	.0347	
Determinations	896	.0149	

[1] Significant at the five percent level.

[2] Significant at the one percent level.

Highly significant differences were detected for both storage periods and varieties, and a significant variety by storage period interaction was encountered. As shown in Table 3 and Figure 1 there was a very definite increase in raffinose content of all varieties tested. The varieties were not, however, responding entirely consistently throughout the storage periods. All varieties showed a significant increase during the first three weeks in storage. After that American No. 2 leveled off and remained constant. The high raffinose selection, 54-410, made a slight but

non significant increase in raffinose content during the third to the sixth week of storage. Selections 54-407 and 54-411 both made a significant increase in raffinose content during the same period. From the sixth to the ninth week only 54-410 made a significant increase in raffinose while 54-407 and 54-411 showed a slight (not significant) decrease. Although some of these selections showed only a slight variation from one storage period to the next the combined effect of the inconsistent responses was great enough to make a significant ($P = .05$) interaction. The interaction effect was, however, very small as compared to the effect of the varieties or storage periods.

The percent increase of raffinose of the two extreme varieties, 54-410 and 54-411, the high and the low raffinose selections respectively, was approximately the same. Selection 54--410 showed an increase of 121.2 percent over the non--stored beets while 54-411 showed a 119.1 percent increase. American No. 2 had the lowest percentage increase of 69.7 percent and 54-407 was very similar with a percent increase of 78.7 percent.

In selecting beets for low raffinose content the plant breeder would obtain a greater spread in his material if he would store the roots for several weeks before they were sampled. Although the increase of raffinose content may be approximately equal on a percentage basis, this additional spread will make the selection of low raffinose beets easier and more accurate.

The curve shown in Figure 1, based on the means for each storage period, resembles a portion of a sigmoid curve. It shows a very rapid increase in raffinose accumulation during the early part of storage and then levels off which is typical for a wide variety of growth phenomena. Oftentimes, these response curves can be adequately approximated, within the limits under investigation, by a simple polynomial. Since the observations during storage were taken at equally spaced intervals the response can be easily characterized by the use of orthogonal polynominals. A linear response was first tested and was found to be significant but the deviations from linearity also were significant. The quadratic component was found to be significant and deviations from the quadratic were non significant. Therefore, this response curve within the limits of interest can be satisfactorily approximated by a second degree polynomial indicating that the rate of raffinose accumulation decreased as the beets remain in storage.

The fact that the raffinose content of topped beets in storage increased indicated that this phenomena is probably tied up with respiration system of the beets. It certainly could not have been associated with the photosynthesis mechanism. The exact

mechanism responsible for raffinose increase is not known and additional investigations are needed to determine it.

One possible explanation or hypothesis could be as follows; as the temperature decreased to near freezing an enzyme may be activated which would convert certain respiratory products into raffinose. Therefore, raffinose is mainly produced during cold weather and when the respiration rate of the beet exceeds the rate of photosynthesis.

As early as 1911 Zitkowski (11) quoted Mr. Kelton, Superintendent of the West Bay City Sugar Company of Michigan, as follows: "All this indicates that raffinose is formed by the action of frost, in this case after the beets were harvested." Zitkowski also reported in the same paper that a "Professor Herzfeld as early as 1889 expressed the opinion that raffinose was formed in larger quantities when beets which had been exposed to freezing weather take on a new growth in which case raffinose was formed from dissolved pectic substances." In this same paper Mr. Zitkowski suggested the possibility of enzymatic action on other carbohydrates which would eventually form raffinose and "one of these conditions was undoubtedly the action of frost."

Perhaps the results of this study have done nothing more than to confirm what Mr. Kelton and Professor Herzfeld knew many years ago. However, in addition the findings have shown that, if a beet goes into storage with a genetically lower raffinose content it will remain lower throughout the storage season.

Summary

The raffinose content of topped beets stored in the root cellar at Rocky Ford, Colorado, showed a significant increase as they remained in storage.

No significant differences for raffinose content were found after a three week period between beets stored in a regular storage pile and those stored in the root cellar.

All varieties showed a significant increase in raffinose content during storage, however, they kept the same ranking, i.e., the varieties which had high and low raffinose content to start with also were the high and low varieties at the end of the experiment.

The quadratic component for storage means was found to be significant, indicating that the rate of raffinose accumulation decreased as the beets remained in storage.

Acknowledgment

The authors gratefully acknowledge the valuable assistance of all members of the Research Department of the American Crystal Sugar Company for their wholehearted cooperation in directing these investigations.

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Effects of Storage on Raffinose Content of Sugar Beets. II. Evaluation of Variance Components for Optimum Sample Size Determination

M. D. Finkner, R. E. Finkner and R. F. Olson[1]

Breeding for improvement of sugar beets in the past has been largely confined to the factors of yield and percent sucrose. The source and extent of experimental and sampling errors associated with the estimation of these two characters have been studied by Immer (5)[2] and Immer and LeClerg (6). Recently however, new techniques, such as flame spectrophotometry and paper chromatography, have been developed which can be used for the determination of many melassigenic substances for mass operation. Very little information is available regarding the magnitude of experimental and sampling errors associated with the use of these new techniques.

The concept of variance components originated with R. A. Fisher who expressed the additive properties of variances in the first edition of his text "Statistical Methods for Research Workers" (4). Early development of variance components as a working tool was made by Yates and Zacopanay (9). The concept of variance components was used in this study to evaluate sources of variation contributing to the sampling and experimental errors associated with the determination of raffinose content in sugar beets by the means of paper chromatography.

Materials and Methods

The materials used in this study were the same as those described previously (3). There were four varieties, four replications and four storage periods arranged in a split plot design. A total of 35 beets of each variety, in each of the four replicacations, were topped and stored in the Rocky Ford, Colorado, root cellar on October 24, 1955. Seven beets of each variety within each replication were selected at random and stored together in crates, or a total of 28 beets per crate. The 16 crates, one crate per each replication and for four different storage dates were randomized and stored in the root cellar at a temperature of approximately 4 degrees Centigrade. The remaining beets, seven roots of each of the four varieties of each replication, were bulked together in large onion sacks and all four sacks,

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[2] Numbers in parentheses refer to literature cited.

each containing a single replication, were buried in the storage pile at Manzanola, Colorado, on the evening of October 25, 1955.

After three weeks the beet samples in the storage pile were taken out, analyzed and compared with samples stored three weeks in the root cellar. Beet samples stored in the root cellar were analyzed at three week intervals. The non-stored check was in storage one day while the experiment was being set up.

Each beet in the storage test was sampled twice by rasping. Each of the two resulting pulp samples was split into half and placed in a plastic sack and frozen. When all beets were rasped the individual samples were taken out of the deep freeze at random and spotted on Whatman filter paper number 1. Determination of raffinose content was similar to the method described by Brown (2) and Serro and Brown (8) and the results are given as percent on dry substance of the beet juice.

Results and Discussion

Table 1 gives the complete analysis of variance and the expected values of mean squares according to Anderson and Bancroft (1) for beets stored in the storage pile compared with beets stored in the root cellar. Variability associated with beets within plots (a sampling error), rasps from the same beet (a sub-sampling error), and determinations (a laboratory error) can be estimated from this analysis. Experimental errors associated with varieties and storage methods can also be estimated from the analysis of variance. Experimental errors will not be considered here however, since it is the usual procedure to base the required number of replications upon some factor (generally yield) which is more variable than a chemical determination.

Table 1.—Analysis of Variance and the Expected Mean Squares of Four Varieties and Two Different Types of Storage for Raffinose Content Expressed as Percent on Dry Substance.

Source of Variation	D.F.	M.S.	Expected Mean Squares
Total	671		
Replications	2[1]	.3237	$\sigma_d^2 + 2\sigma_r^2 + 4\sigma_b^2 + 28\sigma_e^2 + 224\sigma_p^2$
Varieties[2]	3	1.5624	$\sigma_d^2 + 2\sigma_r^2 + 4\sigma_b^2 + 28\sigma_e^2 + 56\sigma_s^2 + 168\Theta_v$
Error A (Rep. x Var.)	6	.1450	$\sigma_d^2 + 2\sigma_r^2 + 4\sigma_b^2 + 28\sigma_e^2 + 56\sigma_s^2$
Storage	1	.1357	$\sigma_d^2 + 2\sigma_r^2 + 4\sigma_b^2 + 28\sigma_e^2 + 336\Theta_s$
Varieties x Storage	3	.2996	$\sigma_d^2 + 2\sigma_r^2 + 4\sigma_b^2 + 28\sigma_e^2 + 84\Theta_{vs}$
Error B	8	.3307	$\sigma_d^2 + 2\sigma_r^2 + 4\sigma_b^2 + 28\sigma_e^2$
Beets within plots	144	.0650	$\sigma_d^2 + 2\sigma_r^2 + 4\sigma_b^2$
Rasping from same beet	168	.0141	$\sigma_d^2 + 2\sigma_r^2$
Determinations	336	.0113	σ_d^2

[1] One replication was lost while removing the beets from the storage pile.

[2] Θ_v, Θ_s, and Θ_{vs} refer to the fixed effects of varieties, storage, and variety by storage interaction, respectively.

Estimates of the components of variance associated with determinations (σ_d^2), raspings (σ_r^2) and beets (σ_b^2) were computed from the expected mean squares of Table 1.

$$(1) \quad \sigma_d^2 = .0113$$

$$(2) \quad \sigma_d^2 + 2\sigma_r^2 = .0141$$

$$2\sigma_r^2 = .0141 - \sigma_d^2$$

$$\sigma_r^2 = \frac{.0141 - .0113}{2}$$

$$\sigma_r^2 = .0014$$

$$(3) \quad \sigma_d^2 + 2\sigma_r^2 + 4\sigma_b^2 = .0650$$

$$4\sigma_b^2 = .0650 - (\sigma_d^2 + 2\sigma_r^2)$$

$$\sigma_b^2 = \frac{.0650 - .0141}{4}$$

$$\sigma_b^2 = .0127$$

Since the sampling scheme for raffinose determination involved the random selection of seven beets per plot, two raspings per beet and two determinations per rasping, the variance of a sample mean on a per determination basis was the mean square for beets within plots divided by the total number of determinations per plot. Also since variance components have additive properties, the variance of a sample mean can be equated to the variance components associated with beets, raspings and determinations. Therefore

$$V_x = \frac{\sigma_d^2}{brd} + \frac{\sigma_r^2}{br} + \frac{\sigma_b^2}{b}$$

$$= \frac{.0113}{28} + \frac{.0014}{14} + \frac{.0127}{7}$$

$$= .00232$$

where V_x is the variance of a sample mean and b, r, and d refer to the number of beets, raspings, and determinations respectively.

Table 2 shows the value of the estimated variance and the percent of the total sampling variability attributed to each of the various components under study.

Table 2.—Estimates of Variance Components Associated with Field Sampling of Beets for Raffinose Determination.

Variance Components		Value	Percent
Determinations	(σd^2)	.0113	44.5
Raspings	(σr^2)	.0014	5.5
Beets	(σb^2)	.0127	50.0
Total[1]		.0254	100.0

[1] Does not include plot variability for either main plots or sub-plots.

The component which exhibited the greatest portion of the total sampling variability was associated with beets within plots. This was to be expected as the varieties used in this investigation were extremely heterozygous. Also, the seven beets were selected at random from each plot allowing for the possible effect of some intra-plot soil variation. The only other component of significance was that associated with the laboratory determinations. This may be interpreted as the failure of the laboratory technique to be completely reproducible.

The analysis of variance and expected values of mean squares for the second phase of the investigation are shown in Table 3. Again only the sampling variability is of particular interest. The sampling scheme for the second phase was identical to that of the first phase although many more plots were involved.

Table 3.—Analysis of Variance and the Expected Mean Squares of Four Varieties and Four Different Storage Dates for Raffinose Content Expressed as Percent on Dry Substance.

Source of Variation	D.F.	M.S.	Expected Mean Squares
Total	1791		
Replications	3	.8649	$\sigma d^2 + 2\sigma r^2 + 4\sigma b^2 + 28\sigma e^2 + 448\sigma p^2$
Varieties	3	4.6281	$\sigma d^2 + 2\sigma r^2 + 4\sigma b^2 + 28\sigma e^2 + 112\sigma a^2 + 448\Theta v$
Error A (Rep. x Var.)	9	.2872	$\sigma d^2 + 2\sigma r^2 + 4\sigma b^2 + 28\sigma e^2 + 112\sigma s^2$
Storage	3	6.7044	$\sigma d^2 + 2\sigma r^2 + 4\sigma b^2 + 28\sigma e^2 + 448\Theta s$
Varieties x Storage	9	.2812	$\sigma d^2 + 2\sigma r^2 + 4\sigma b^2 + 28\sigma e^2 + 112\Theta vs$
Error B	36	.1082	$\sigma d^2 + 2\sigma r^2 + 4\sigma b^2 + 28\sigma e^2$
Beets within plots	384	.1156	$\sigma d^2 + 2\sigma r^2 + 4\sigma b^2$
Rasping from same beet	448	.0347	$\sigma d^2 + 2\sigma r^2$
Determinations	896	.0149	σd^2

[1] Θv, Θs, and Θvs refer to the fixed effects of varieties, storage, and variety by storage interaction, respectively.

Estimates of the variance components, computed as previously shown, and the percent of the total sampling variability attributed to each are listed in Table 4.

Table 4.—Estimates of Variance Components Associated with Field Sampling of Beets for Raffinose Determination.

Variance Components		Value	Percent
Determinations	(σd^2)	.0149	33.1
Raspings	(σr^2)	.0099	22.0
Beets	(σb^2)	.0202	44.9
Total[1]		.0450	100.0

[1] Does not include plot variability for either main plots or sub-plots.

Again the greatest variance was from beets within plots which contributed about 45% of the total sampling error. Following it was the component for laboratory determinations. Although the component of variance associated with the raspings was somewhat larger in this case, contributing 22 percent of the total sampling error, it was not significant at the .05 level of probability.

The variance of a sample mean for this phase was estimated as follows:

$$V_{\bar{x}} = \frac{\sigma_d^2}{b\,r\,d} + \frac{\sigma_r^2}{b\,r} + \frac{\sigma_b^2}{b}$$

$$= \frac{.0149}{28} + \frac{.0099}{14} + \frac{.0202}{7}$$

$$= .00413$$

The variance of a sample mean can be reduced to any value desirable by merely increasing the sampling intensity. Disregarding cost the most efficient method of reducing the variance of a sample mean would be to increase the number of beets per plot. Not only was the beet-to-beet variability the greatest of the three sampling components, but the number of beets appeared in the denominator of all three components.

It was also apparent that it was unnecessary to rasp each beet more than once. Therefore, if one should double the number of beets per plot, rasp each beet once and run duplicate determinations, the total number of chemical determinations would be the same as before. The variance of a sample mean, however, would be as follows:

$$V_{\bar{x}} = \frac{.0149}{28} + \frac{.0099}{14} + \frac{.0202}{14}$$

$$= .00268$$

This would reduce the variance of a sample mean 35 percent without requiring any additional chemical determinations. Considering the same sampling pattern for the first phase of the experiment the variance would have been .00141, a reduction of 39 percent.

A primary objective in the statistical design of such a sampling procedure is to mininize the cost of obtaining the sample estimate if the desired degree of precision is fixed, or conversely, to maximize the precision of the estimate obtained from a given amount of expenditure including personnel, time and equipment. If the cost per sampling unit is known or can be accurately estimated, and if the cost is proportional to the number of units sampled at each level, the optimum allocation of the sampling units can be determined. Marcuse (7) has developed cost functions for a three stage sampling pattern such as the one de-

scribed in this paper. Therefore the optimum allocation of sampling units for a minimum cost subject to the constraint that the allowable amount of variance is preassigned is as follows:

$$b' = \frac{\sigma_b}{V} \frac{\sum_{i=b,r,d} (\sigma_i \sqrt{c_i})}{\sqrt{c_b}}$$

$$r' = \frac{\sigma_r}{\sigma_b} \sqrt{\frac{c_b}{c_r}}$$

$$d' = \frac{\sigma_d}{\sigma_r} \sqrt{\frac{c_r}{c_d}}$$

where v is the preassigned variance, σ_b, σ_r, and σ_d are the standard deviations associated with beets, raspings, and determinations respectively; and c_b, c_r, and c_d are the unit costs for beets, raspings, and determinations respectively.

Likewise Marcuse (7) has shown that the optimum allocation when the total amount of cost is fixed is:

$$b'' = \frac{\sigma_b}{\sum_{i=b,r,d} (\sigma_i \sqrt{c_i})} \frac{c}{\sqrt{c_b}}$$

$$r'' = \frac{\sigma_r}{\sigma_b} \sqrt{\frac{c_b}{c_r}}$$

$$d'' = \frac{\sigma_d}{\sigma_r} \sqrt{\frac{c_r}{c_d}}$$

where c is the total allowable cost and the other notations are the same as above.

Cost information for the sampling procedure of these experiments was obtained from actual expenditures for labor and materials. (Expenditures such as capital outlay, taxes, etc. were not included). The cost per beet for harvesting, labeling and storing was approximately twenty cents ($0.20). The cost for a single rasping was determined to be about ten cents ($0.10). The cost per single determination of raffinose including calculations necessary to obtain a proper value based on dry substance was approximately twenty cents ($0.20).

Since the sampling procedures for both of the experiments reported in this paper were identical, the best available estimates of the variance components were the weighted averages of the

components from the two experiments. The proper weighing factor was the degrees of freedom associated with each component.

Therefore estimates of the variance components for the three sampling stages are:

$$s_d^2 = .0139 \quad (s_d = .1179)$$
$$s_r^2 = .0076 \quad (s_r = .0872)$$
$$s_b^2 = .0182 \quad (s_b = .1349)$$

Using these estimates along with the cost information that $c_d = .20$, $c_r = .10$, and $c_b = .20$ optimum allocation of sampling units can be obtained. Assuming that a variance of a sample mean of .0025 ($s_x = .05$) will provide adequate precision, the sampling units for each stage were computed as follows:

$$b' = \frac{s_b}{V} \frac{\sum_{i=b,r,d} (s_i \sqrt{c_i})}{\sqrt{c_b}}$$

$$= \frac{.1349}{.0025}\left[\frac{(.1349)\ (.4472) + (.0872)\ (.3162) + (.1179)\ (.4472)}{.4472}\right]$$

$$= \left[\frac{.1349}{.0025}\right]\left[\frac{.1406}{.4472}\right]$$

$$= \frac{.01897}{.00112}$$

$$= 16.9$$

$$r' = \frac{s_r}{s_b}\sqrt{\frac{c_b}{c_r}}$$

$$= \left[\frac{.0872}{.1349}\right]\left[\frac{.4472}{.3162}\right]$$

$$= \frac{.03900}{.04266}$$

$$= 0.91$$

$$d' = \frac{s_d}{s_r}\sqrt{\frac{c_r}{c_d}}$$

$$= \left[\frac{.1179}{.0872}\right]\left[\frac{.3162}{.4472}\right]$$

$$= \frac{.03728}{.03900}$$

$$= 0.96$$

Obviously, the sampling units must be whole numbers. Therefore, the optimum sampling procedure is 17 beets per plot, one rasping per beet, and one chemical determination per rasping. This would be accomplished at a total cost of $8.50 per plot which is minimum for these conditions.

Under some conditions it would be desirable to determine the optimum sampling scheme for a fixed cost. Suppose it has been determined that sampling costs must not exceed $5.00 per plot.

Computations for optimum allocations of sampling units which will provide a minimum variance were as follows:

$$b'' = \frac{S_b}{\sum_{i=b,r,d} (s_i \sqrt{c_i})} \quad \frac{C}{\sqrt{c_b}}$$

$$= \left[\frac{.1349}{(.1349)\ (.4472) + (.0872)\ (.3162) + (.1179)\ (.4472)}\right]\left[\frac{5.00}{.4472}\right]$$

$$= \left[\frac{.1349}{.1406}\right]\left[\frac{5.00}{.4472}\right]$$

$$= \frac{.6745}{.0629}$$

$$= 10.7$$

$$r'' = \frac{S_r}{S_b} \sqrt{\frac{c_b}{c_r}}$$

$$= \left[\frac{.0872}{.1349}\right]\left[\frac{.4472}{.3162}\right]$$

$$= \frac{.03900}{.04266}$$

$$= 0.91$$

$$d'' = \frac{S_d}{S_r} \sqrt{\frac{c_r}{c_d}}$$

$$= \left[\frac{.1179}{.0872}\right]\left[\frac{.3162}{.4472}\right]$$

$$= \frac{.03728}{.03900}$$

$$= 0.96$$

Again each of the sampling intensities must be rounded to a whole number. Since r″ and d″ must be rounded to one, it was necessary to round b″ to 10 in order to satisfy the requirement that the cost not exceed $5.00 per plot. The minimum variance for this sampling pattern is .0040.

It is worthy of note that, except for the first level (beets), the optimum combination of the number of sampling units is independent of the given degree of precision or the fixed total cost. Therefore, when planning an experiment using nested sampling in three stages one needs to be concerned with the given cost or precision only in selecting the number of primary sampling units.

Summary

The concept of variance components was used to evaluate sources of variation contributing to sampling errors associated with the determination of raffinose content in sugar beets by means of paper chromatography. Two experiments were involved, each of which utilized identical three stage nested sampling procedures. Weighted averages of the estimated components of variance were .0182, 0076, and .0139 for beets, raspings, and chemical determinations respectively.

Actual costs were determined to be twenty cents ($0.20) per beet, ten cents ($0.10) per rasping, and twenty cents ($0.20) per chemical determination. Using cost functions developed by Marcuse (7) the optimum allocations of sampling units for a preassigned variance of .0025 is 17 beets per plot, one rasping per beet and one chemical determination per rasping. The total cost resulting from this sampling procedure is $8.50 per plot. Assuming that the total cost per plot must not exceed $5.00 the optimum allocation of sampling units is 10 beets, one rasping, and one determination. The variance of the sample mean would then be .0040.

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Response of Sugar Beets to Nitrogen Depletion In Relation to Root Size[1]

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Received for publication October 13, 1958

Introduction

Sugar beet yields have been increasing and sucrose concentrations decreasing in California for over 20 years (14)[3]. Increased plant populations, improved varieties and better production practices have been largely responsible for the higher yields. Lower sucrose concentration has often been associated with large beets, and higher sucrose concentrations with small beets. Whether lower sucrose concentration is inherent in large beets or results from some conditions that lead to large size is a problem of considerable interest.

Increased use of nitrogenous fertilizers has been a major factor contributing to higher yields. Continued vegetative growth under high nitrogen conditions, however, is known to be incompatible with high sucrose at harvest time (12), and the low sucrose values may be symptomatic of too much nitrogen. The observed inverse correlations between beet weight and sucrose concentration may be due to large and small beets responding differently to the normal preharvest period of nitrogen deficiency. Under the influence of high nutrition and a constant environment, the size of beet tops appears to be largely independent of root size (12). If this is the case, then one effect of nitrogen deficiency in reducing growth would be to leave small beets with the same effective leaf area for photosynthesis as large beets. Thus, for a given period of nitrogen deficiency, the greatest increase in sucrose concentration should be noted in small beets, because of greater tops relative to root size.

In the present series of experiments, the response of sugar beets to nitrogen deficiency in relation to root size was investigated with nutrient culture techniques at Berkeley and Davis, California. The primary advantage of the nutrient culture technique in this study was that it provided an easy means of controlling nitrogen nutrition precisely and providing adequate amounts of all other nutrients known to be essential to plant growth. Root size was varied by planting beets on three different dates. This appeared to be a satisfactory method for controlling root size because, after top development was complete,

[1] This study was supported in part by a grant from the beet sugar companies operating in California and the California Beet Growers' Association, Ltd.

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[3] Numbers in parentheses refer to literature cited.

the size and character of the tops would be determined by climate and nutrition (10, 12, 17), and the only major residual effect of the various planting dates would be in root size. The experiments as designed provided information on the main effects of root size (planting date) in relation to nitrogen depletion, and not on environmental variables that would influence root size under field conditions.

Methods

Duplicate experiments, using, as nearly as possible, the same materials and procedures, were conducted under natural environmental conditions at Berkeley and Davis in 1957. General procedures for such pot studies have been described (17).

The pots used were ten-gallon carbide cans (32 cm. diameter, 52 cm. height) painted inside with Amercoat No. 33 protective coating and outside with aluminum paint. Drainage was provided by drilling four 0.25-inch holes equidistant on the sides near the bottom of the pots. The pots were filled with No. 2 grade vermiculite as a rooting medium, and firmly settled. Sugar beet seed (variety US 75) treated with Phygon XL at the rate of one percent was planted by placing ten seedballs equidistantly in a circle (diameter 23.5 cm.) and pressing the seeds into the vermiculite to a depth of 1.9 cm. The pots were spaced in rows with a minimum of 50 cm. between cans in the growing area (concrete slab at Berkeley; gravel at Davis). After emergence, the beets were thinned periodically, retaining uniformly sized vigorous plants until at the 6 to 8 leaf stage, only two plants remained per pot. Two plants give considerably greater precision than one (15).

The pots were watered daily with complete nutrient solution, a modified half-strength Hoagland solution No. 1 prepared with tap water (18). On the nitrogen cut-off date the pots were heavily leached with tap water to remove the bulk of the residual nitrogen from the vermiculite, and the minus-nitrogen pots subsequently received a solution free of added nitrogen (Table 1).

Three mean root-size classifications were obtained by planting 26 pots on each of three dates: March 1, May 1, and June 1.

Table 1.—Composition of the Modified Half-Strength Hoagland No. 1 Nutrient Solutions.

Solution	Millimoles of Salt per Liter of Nutrient				
+N	0.5 KH_2PO_4	1.0 $MgSO_4$-7 H_2O	2.5 KNO_3	2.5 $Ca(NO_3)2$	0.5 Na Cl
—N	0.5 KH_2PO_4	1.0 $MgSO_4$-7 H_2O	2.5 K_2SO_4	2.5 Ca Cl_2-2 H_2O	0.5 Na Cl

Minor elements were added to both solutions to give a final concentration, expressed in milligrams per liter, as follows: 0.25 B, 0.25 Mn, 0.02 Zn, 0.01 Cu, 0.005 Mo, and 2.5 Fe.

On August 15, six pots from each group were harvested and the remaining 20 pots were divided into two groups of ten pots each. One of these groups continued to receive the standard solution while the other was watered with the minus-nitrogen solution. All remaining pots were harvested on October 15.

Old leaves (50 percent or more of blade dead) were gathered periodically during the growing season. These were dried so that total leaf production could be determined. Just before harvest, measurements were made of the height of tallest recently matured leaf, and the diameter of the foliage canopy. At harvest, the tops were separated from the roots at the oldest living leaf and the following measurements were obtained: (a) total fresh and dry weight of green tops; (b) total weight of the individual roots plus crown, and (c) separate weights of the crown (portion between the upper and lower leaf scar) and of the storage roots. For NO_3-N analyses (5), petioles of ten recently matured leaves were collected from each pot on the harvest dates and on September 15 (two petioles per pot). Pulp samples from individual roots and also on October 15, from crowns of beets planted on March 1, were prepared with a Kiel rasp, and analyzed for sucrose in duplicate by the method of Browne and Zerban (1). Total beet weight (roots and crowns) per pot and weighted mean sucrose percentage are reported in the results. Weighted mean sucrose percent was calculated by dividing total sucrose per pot (sum of the products of individual beet weights and sucrose percentages) by total beet weight per pot.

Results

The Berkeley and Davis harvest results are summarized separately in Tables 2 and 3.

A satisfactory range of mean root sizes were obtained by August 15 at both locations. The later planting dates, however, had not attained full top development, as shown by significant date effects on fresh and dry weight of tops at both locations and on top diameter at Davis. Immature top development was reflected in significant date effects on weighted mean sucrose percentage at both locations: small beets with small tops had the lowest concentrations of sucrose. This agrees with previous observations (12) that top growth has priority over other growth for sucrose utilization.

The failure to obtain equal top development from all plantings at the nitrogen cutoff date slightly complicates interpretation of the changes that took place between August 15 and October 15. The changes in root weight and sucrose percentage

Table 2.—Harvest Results From Berkeley for Studies of Nitrogen Depletion in Relation to Root Size, 1957. All Values on a per Pot Basis: Means of 6 Pots for August 15; Means of 10 Pots for October 15.

Harvest Date	N Level	Planting Date	Beet Root Plus Crown				Tops			
			Weight gm.	% Crown	Sucrose gm.	Sucrose %	F.W. gm.	D.W. gm.	Height cm.	Diameter cm.
August 15	+ N	March 1	3 520	9.3	421	12.0	2,618	268	45.3	73.0
		May 1	1,910	7.6	214	11.0	1,947	196	41.8	69.9
		June 1	780	1.9	77	9.9	1,340	109	44.3	70.0
		$\bar{x}$	2,070	6.3	237	11.0	1,970	191	43.8	71.0
	LSR_{01}[1] (p = 3)		770	3.9	88	1.1	500	56	7.9	8.6
	F (Planting)		60.6[4]	19.4[4]	65.7[4]	18.9[4]	31.8[4]	37.9[4]	0.89 NS	0.81 NS
	Error M.S. (15 df)		187,400	4.69	2,669	0.3449	77,070	1,001	21.73	23.37
	C.V. (%)[2]		20.9	34.6	21.7	5.3	14.1	16.6	10.6	6.8
October 15	+ N	March 1	5,210	16.8	614	11.8	2,240	238	42.3	69.5
		May 1	3,870	12.9	464	12.0	2,480	267	45.6	70.9
		June 1	2,650	12.1	306	11.5	2,120	218	45.8	70.4
	+ N	$\bar{x}$	3,910	13.9	461	11.8	2,280	241	44.6	70.3
	— N	March 1	5,230	14.9	769	14.7	890	111	26.1	62.3
		May 1	2,920	13.3	448	15.3	730	91	26.6	61.0
		June 1	1,960	8.9	329	16.9	670	87	26.6	66.3
	— N	$\bar{x}$	3,370	12.4	515	15.6	763	96	26.4	63.2
	LSR_{01}[1] (p = 6)		920	5.2	141	1.1	760	70	3.3	9.8
	F (Planting)		90.0[4]	9.28[4]	63.4[4]	6.89[4]	0.78 NS	1.49 NS	3.62[3]	0.73 NS
	F (Nitrogen)		9.00[4]	2.37 NS	3.95 NS	334.0[4]	106.0[4]	113.3[4]	796.5[4]	13.7[4]
	F (P x N)		2.64 NS	1.01 NS	3.58[3]	13.6[4]	0.69 NS	1.33 NS	1.94 NS	0.77 NS
	Error M.S. (54 df)		480,700	15.13	11,280	0.6765	325,100	2,768	6.056	54.26
	C.V. (%)		19.0	29.6	21.8	6.0	37.5	31.2	7.0	11.0

[1] LSR_{01}: Least significant range for the 1% level for random comparisons among p means (3).

[2] Coefficient of variability: (s/x)100.

[3] Significant at the .05 level.

[4] Significant at the .01 level.

NS = Not significant.

NS = Not significant.

Table 3.—Harvest Results from Davis for Studies of Nitrogen Depletion in Relation to Root Size, 1957. All Values on a Per Pot Basis: Means of 6 Pots for August 15; Means of 10 Pots for October 15.

			Beet Root Plus Crown				Tops			
Harvest Date	N Level	Planting Date	Weight gm.	% Crown	Sucrose gm.	Sucrose %	F.W. gm.	D.W. gm.	Height cm.	Diameter cm.
August 15	+ N	March 1	2,750	4.4	353	12.9	1,460	202	33.5	67.7
		May 1	1,310	1.0	157	12.0	962	128	37.0	70.8
		June 1	550	0.0	59	10.8	556	71	35.9	60.3
		$\bar{x}$	1,540	1.8	189	11.9	993	134	35.5	66.3
	$LSR_{.05}$ (p = 3)		330	3.6	36	1.3	393	55	4.6	7.6
	F (Planting)		221.6[a]	7.80[a]	337.3[a]	12.9[a]	25.3[a]	27.2[a]	2.87 NS	9.34[a]
	Error M.S. (15 df)		33,740	4.058	398.6	0.493	48,450	951.7	6.687	18.40
	C.V. (%)		11.9	112.0	10.5	5.9	22.2	23.1	7.2	6.5
October	+ N	March 1	4,390	14.2	468	10.7	1,780	200	32.6	60.2
		May 1	3,380	10.3	365	10.8	1,840	195	36..7	64.6
		June 1	2,710	6.1	284	10.5	1,720	179	35.8	66.6
	+ N	$\bar{x}$	3,490	10.2	360	10.7	1,780	191	35.0	63.8
	— N	March 1	3,670	7.8	511	13.9	780	116	22.0	49.2
		May 1	2,480	7.7	408	16.5	600	87	25.0	54.6
		June 1	1,600	2.3	273	17.1	410	62	25.6	59.2
	— N	$\bar{x}$	2,580	5.9	397	15.8	600	88	24.2	54.3
	$LSR_{.05}$ (p = 6)		510	4.4	70	1.3	410	57	6.1	8.1
	F (Planting)		121.8[a]	21.9[a]	81.2[a]	15.5[a]	2.65 NS	4.75[a]	3.69[a]	9.17[a]
	F (Nitrogen)		85.1[a]	24.6[a]	3.45 NS	455.5[a]	214.3[a]	108.4[a]	82.2[a]	36.5[a]
	F (P x N)		1.04 NS	1.61 NS	1.84 NS	16.7[a]	1.44[a]	1.03 NS	0.13 NS	0.46 NS
	Error M.S. (54 df)		56,130,000	11.11	2.734	0.8813	98,060	1,468	21.43	36.86
	C.V. (%)		12.6	41.3	13.6	7.1	26.3	27.4	15.3	10.2

For explanation of table, see footnotes to table 2.

for these later plantings are probably smaller for the nitrogen-deficient plants than could have been obtained had top size been equal on August 15 for all plantings.

The beets responded rapidly when watered with the minus-nitrogen solution after August 15. Within seven days, yellowing was apparent and immature leaves stopped growing. On September 15 and October 15, the minus-nitrogen beets showed petiole NO_3-N levels indicative of extreme nitrogen deficiency (Table 4). The tops of the low-nitrogen beets had small-bladed, short-petioled, yellow leaves in flattened rosettes typical of nitrogen-deficient plants. It thus appeared that nitrogen cutoff was rather sharp, not a slow transition, and that the nitrogen-deficient period before the October 15 harvest was longer than seven weeks.

Table 4.—Nitrate-Nitrogen Content (p.p.m.; Dry Basis) of Petioles of Recently Matured Leaves[1].

Sampling Date	Location[2]	N Level	Planting Date			Mean[3]
			March 1	May 1	June 1	
Aug. 15	B	High	9,760	9,530	9,720	9,670
	D	High	9,270	7,960	9,640	8,960
Sept. 15	B	High	8,670	8,160	8,810	8,540
	D	High	6,440	6,660	7,180	6,760
	B	Low	385	242	212	280
	D	Low	308	333	121	254
Oct. 15	B	High	5,840	5,930	7,360	6,380
	D	High	8,110	6,680	9,340	8,040
	B	Low	268	236	192	232
	D	Low	544	292	142	326

[1] Critical level: 1000 p.p.m.

[2] B (Berkeley); D (Davis).

[3] On October 15, at Berkeley, high N value for June 1 is significantly different (.05 level) from the means of the other two planting dates; no other planting date effects are significant. All differences between high and low N at one location and one sampling date are highly significant (.01 level).

By October 15 at both locations, total beet root weight had increased about one kilogram per pot in the minus-nitrogen series and nearly two kilograms per pot with full nutrition. The interactions between planting date and nitrogen level were not significant, though total beet weights for the March 1 planting at Berkeley showed the same increases as both nitrogen levels. This may indicate that pot size limited the growth of the plus-nitrogen beets in that instance.

The crown percentage of total beet weight was highest with the large, early-planted beets, and plus-nitrogen beets tended to have a larger percentage of crown than nitrogen-deficient

beets. This was related to a greater old-leaf production (not shown in tables) for the early plantings.

The March 1 plus-nitrogen tops showed little change in size between August 15 and October 15. During that period the later plantings attained full top development, equalled the March 1 beets by October 15. Careful examination revealed slightly larger leaf size and lighter color in the late plantings. Fresh and dry weights, heights and diameters were rather uniform within each location, but the Berkeley plants had greater top development as well as larger roots. Large differences between nitrogen levels were apparent in all top measurements except diameter—the relatively small decrease in top diameter with nitrogen deficiency was indicative of the rosette habit. The decrease in top weight in the minus-nitrogen series with later planting was not significant (except as measured by dry weight at Davis), thus indicating that the nitrogen-deficient plants also tended toward a uniform top size at each location.

Weighted mean sucrose percent on October 15 was uniformly low with high nitrogen for all planting dates at both locations (Tables 2 and 3). These values were slightly different from those obtained on August 15 in two respects: (a) the late-planted beets no longer showed lower values than the early-planted beets, and (b) the early-planted beets showed a lower sucrose percentage at Davis (12.9% compared to 10.7%) and no significant change at Berkeley (12.0% compared to 11.8%). The nitrogen-deficient beets for all planting dates showed marked increases in sucrose concentration over those of plus-nitrogen beets for both August 15 and October 15. The highest values, 17.1 percent at Davis and 16.9 percent at Berkeley, were obtained with the small June 1 beets, while March 1 plantings yielded the lowest sucrose percentages of the series, 13.9 percent at Davis and 14.7 percent at Berkeley. All these values were significantly greater than in the plus-nitrogen series.

Sucrose yields from minus-nitrogen beets tended to be slightly higher than from the plus-nitrogen beets, especially with the March 1 planting (significant only for Berkeley, at 5 percent level). Thus the increased sucrose percentage in the roots more than offset the reduced growth of the minus-nitrogen plants. As expected, sucrose yield declined with later planting.

Weighted mean sucrose percentage in the crowns was of interest, but only the March 1 beets had crowns that were large enough by October 15 to permit sampling for sucrose determination (Table 5). The sucrose percentage of crowns and roots of the plus-nitrogen plants was not greatly different (roots greater than crowns; significant, 5 percent level, only, at Berk-

Table 5.—Weighted Mean Sucrose Percentages of Crowns and Roots on October 15 for the March 1 Planting. All Values on a per Pot Basis: Means of 10 Pots.

N Level	Part	Location	
		Berkeley	Davis
+ N	Crown	10.3	10.5
	Root	11.8	10.7
— N	Crown	12.5	12.7
	Root	14.7	13.9
	$LSR_{.05}$ ($p = 4$)	1.3	1.0
	$LSR_{.01}$ ($p = 4$)	1.8	1.3
	F (Nitrogen)	34.4[a]	77.2[a]
	F (Part)	18.4[a]	4.95[a]
	F (N x P)	0.82 NS	2.72 NS
	Error M.S. (36 df)	1.877	0.9763
	C.V. (%)	11.1	8.3

For explanation of table, see footnotes to Table 2.

eley) while under minus-nitrogen conditions crowns contained significantly lower sucrose concentrations than roots. Sucrose levels were higher in the minus-nitrogen plants in both the crowns and roots.

Ulrich (12), with beets grown in nutrient culture under high nitrogen conditions in a controlled-temperature greenhouse, also found that root mean sucrose did not differ greatly from crown mean sucrose during the first season of growth. Considering the entire beet, root plus crown, it appears that under high nitrogen conditions, the over-all sucrose percent is not appreciably influenced by the presence of the crown. Under low nitrogen conditions the presence of crown tissue contributes to a lower sucrose percent for the whole beet.

Discussion

The means for beet root weight and weighted mean sucrose percent for the minus-nitrogen series show a clear inverse correlation; small beets had high sucrose levels. The increases in sucrose percentages of the minus-nitrogen plants over the plus-nitrogen plants were rather large and, in terms of sucrose produced, more than compensated for the lower beet yields. The net carbon assimilation rates were apparently nearly equal for the seven-week interval of nitrogen deficiency in both series, since the very slightly lower yields of sucrose in the plus-nitrogen group appear to have been offset by greater weights of roots and total tops. This indicates that sucrose yields would prob-

ably also have been equal for shorter intervals of nitrogen deficiency. There is an upper limit to the sucrose concentration that the sugar beet plant can attain, and the rate of accumulation probably declines as this limit is approached. Large beets, from the data presented here, approach that limit more slowly and would require a longer period of nitrogen deficiency to attain a given sucrose level. A longer period of nitrogen deficiency for large beets, however, might be subject to the hazards of further loss of tops and hence reduced carbon assimilation rates.

It is worth nothing that the more rapid increase in sucrose percent in small roots with nitrogen deficiency may indicate that the response in sucrose percent to any change in the environment would be more rapid in small roots than in large roots. Thus a change from warm to cool climate or from long to short days would be reflected more quickly by the small roots. This may account in part for Ulrich's report (15) of higher sucrose concentrations in small, crowded plants than in large, uncrowded plants.

The uniform sucrose concentrations observed under high nitrogen conditions for all planting dates are of interest since this agrees with the concept of an equilibrium sucrose value determined primarily by climate (particularly night temperature) and variety (11, 13, 17). Sucrose percentages for high-nitrogen beets planted on March 1 at Berkeley were nearly equal on the two harvest dates, and accumulated temperature sums for 28 days prior to harvest (Table 6) also showed little change. At Davis, however, with comparable minimum temperatures, there was a noticeable decline in sucrose percentage from August 15 to October 15. Day lengths at the two locations were nearly equal, but light intensity and maximum temperatures might explain the discrepancy. Davis normally has higher light intensities than Berkeley (16), but in 1957 there were ten days

Table 6.—Minimum Night and Maximum Day Temperatures above 32° F. Summed Daily for Four Weeks Prior to Harvest.

Harvest Date	Temperature	Location	
		Berkeley	Davis
August 15	Minimum	663	635
	Maximum	1,150	1,820
October 15	Minimum	709	572
	Maximum	1,160	1,340

of partial or complete overcast in late September and October, and the low light intensities may have contributed to lower sucrose values. A further possibility is the occurrence of higher maximum temperatures prior to August 15. The resultant temporary daily wilting may have reduced vegetative growth, thus accounting for some of the differences.

The goal in the commercial production of sugar beets is a high yield of sucrose. A high yield of roots containing a low percentage of sucrose obtained under high-nitrogen conditions has several disadvantages: to the grower there is an increased cost of fertilizing and harvesting; and to the processor, a low processing quality. Thus, economic sucrose production with present varieties is partially dependent on a period of nitrogen deficiency prior to harvest. The present experiments indicate that the net result of a nitrogen deficiency period is dependent on beet root size.

In applying these results to field conditions, several things need to be considered. Until sugar beet varieties are available that will "ripen" naturally to a high sucrose concentration under high-nitrogen conditions, it may be possible for the grower to take advantage of the knowledge that small beets respond more rapidly than large beets to changes in nitrogen status. It would not be practical for a grower to reduce the mean root size by delaying planting date. Two obvious alternatives remain: (a) reduce the average plant spacing, thus increasing the plant population, and (b) alter the length of the period of nitrogen deficiency prior to harvest.

The results reported here do not bear directly on either of these alternatives, although, considering the first alternative, most evidence (2) indicates that maximum beet yields are obtained by a wide range of populations as long as enough plants are grown to canopy the available space completely with leaves at an early date, yet not so many that small, unmarketable roots result. Within this range, top development per plant will be influenced by competition from adjacent plants for heat, light, and carbon dioxide as well as water and nutrients from the soil. Thus, under field conditions, small, crowded plants would not have the same size of tops as larger, uncrowded plants, but proportionally, top development for crowded plants would be greater than root development (15), tops having priority over storage root growth. Under such conditions, the effects of nitrogen deficiency in relation to root size might be greater or less than in the present experiments. An inverse correlation between beet root size and sucrose percentage in the field might still be anticipated.

The second alternative also appears reasonable since sucrose yields for a planting date were nearly the same at both nitrogen levels after seven weeks of nitrogen deficiency. However, as pointed out earlier, hazards might be encountered with longer periods of nitrogen deficiency. Fields with large beet roots (high yields and/or low populations) could be expected to have lower sucrose percent than fields with small roots (low yields and/or high populations) unless they were allowed longer intervals of nitrogen deficiency. If the small roots result from some factor such as disease, then this relationship might not be valid.

Negative correlations between sucrose percent and beet weight have been frequently reported for field-grown beets (4, 8, 9), though this is not always the case (e.g., significant positive correlations may be calculated for the data of Hills reported by McGinnis (7)). In previous work (unpublished) with beets grown under high-nitrogen nutrition in nutrient culture, no correlation was found between beet root weight and sucrose percent within a variety. Comparisons between varieties were not made.

In the present study, correlation coefficients (Table 7) for the October 15 harvest were calculated for each treatment. Per-pot values were used rather than those of individual beets because of the association (competition, exposure, etc.) between the two beets in the same pot. Correlations computed from individual root values show more significant values (positive and negative), but the conclusions are the same.

Table 7.—Correlation Coefficients for Weighted Mean Sucrose Percentage Versus Total Beet Weight per Pot for the October 15 Harvest (n = 10).

N Level	Planting Date	Location	
		Berkeley	Davis
+ N	March 1	+ 0.181	— 0.141
	May 1	— 0.055	— 0.261
	June 1	+ 0.378	— 0.352
— N	March 1	+ 0.138	+ 0.030
	May 1	+ 0.584	— 0.222
	June 1	— 0.643	— 0.326

Required r-values (n = 10): $r_{.05} = 0.602$ $r_{.01} = 0.735$

Considering the values in Table 7, the only significant correlation (negative) was with the June 1 minus-nitrogen beets at Berkeley: the May 1 minus-nitrogen beets at the same location approached a significant positive value. Thus the most likely conclusion from these correlations is that the open-pollinated

variety, US 75, over a wide range of root sizes and nitrogen nutrition conditions, shows little or no genotypic (within a treatment) correlation between beet root size and sucrose percent. It may be possible that the selective thinning for uniform vigorous plants reduced the genetic variability for root size. However, seedling stands were generally quite uniform and the only obvious selection pressure was against late-germinating plants and plants weakened by seedling diseases.

The inverse relationship between beet root size and sucrose percent shown in these experiments by the minus-nitrogen plants is an environmental, or phenotypic (6), correlation. With high nutrition, the highest sucrose values observed under favorable environmental conditions (cool nights and long days) are about 16 percent (17). Thus, under field conditions, when using high sucrose percent, say 16 percent or over as a selection base, the plants selected may have merely been situated in a favorable micro-environment for the phenotypic expression of high sucrose. Appreciable genetic variability may exist relating to the response of the sugar beet plant to cool temperatures and nitrogen deficiency but other environmental effects may be so variable under field conditions that such selections, though desirable, would have low heritability.

The results reported here indicate that one way to reduce environmental variability appreciably, and hence increase heritability, would be to avoid any degree of nitrogen deficiency among the plants to be selected for high sucrose percent. If it appears desirable to select under "normal field conditions," i.e., deficient nitrogen at harvest time, sucrose percent will have little meaning unless the degree and length of the nitrogen deficiency is uniform for all plants. Such uniformity cannot be achieved in the field, and appears possible only in nutrient culture. Since for any one planting date sucrose yields were equal at both nitrogen levels, a low nitrogen selection might be made more effectively on the basis of sucrose yields (8) than on sucrose percentages alone.

Summary

The responses of sugar beets to nitrogen depletion in relation to root size were investigated outdoors at Berkeley and Davis, California, using ten-gallon cans with vermiculite and nutrient solutions as a culture medium. Three root size classifications were obtained by planting beets on three different dates: March 1, May 1, and June 1. All plants were watered daily with a complete nutrient solution until August 15. Then one group of pots continued to receive complete solution while the other

group was watered with a solution lacking nitrogen. Harvests were made on August 15 and October 15.

On October 15, plus-nitrogen beets, regardless of root size (planting date), had uniform top development and sucrose concentration at each location. The minus-nitrogen plants had smaller (typical nitrogen-deficient type), tops and lower root yields than the plus-nitrogen plants. Sucrose concentrations, however, were much higher and showed a striking inverse relationship to mean root size. Sucrose yields, however, were about equal for plus- and minus-nitrogen beets. Starting from a high-nitrogen status, small beets increased faster in sucrose concentration with the onset of nitrogen deficiency than did large roots.

Under plus-nitrogen conditions, sucrose percentages in the crowns were slightly less than in roots. Under minus-nitrogen conditions, sucrose concentrations increased in both roots and crowns, with the largest increases observed for roots.

Within each treatment (planting date x nitrogen level combination) there was no evident correlation between sucrose concentration and beet root weight, indicating that such correlations when observed are largely environmental responses and not inherent properties of large or small roots.

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Influence of the Seedball on Speed of Germination of Sugar Beet Seeds[1]

F. W. Snyder[2]

Erratic germination of sugar-beet seeds is rather common. Some varieties and progenies germinate more rapidly than others (5, 6)[3]. Inhibitory substances present in the seedball have been shown to delay or inhibit germination (1, 4, 8). In contrast, the author has observed in certain progenies of sugar beets that the seedballs do not retard germination but actually stimulate germination and early growth of seedlings. The structure of the seedball might also be expected to influence speed of germination. This is a report of observations from experiments designed to evaluate the effect of some seedball characteristics on germination.

Methods and Materials

The germination tests were conducted in closed plastic boxes at room temperature. The piece of saran plastic screen in each box was supported on five pieces of glass tubing. Twenty or twenty-five seedballs were placed in each quarter of the box in contact with the surface of approximately 180 milliliters of water or nutrient solution (Figure 1). The molar composition of the nutrient solution was as follows: $CaCl_2$, 0.0255; $Ca(NO_3)_2 \cdot 4H_2O$, 0.0040; KCl, 0.0445; KH_2PO_4, 0.0740; $MgSO_4 \cdot 7H_2O$, 0.0240; Na_2SO_4, 0.0295. The osmotic pressure of this solution is 10.1 atmospheres. This method of germination is designated the liquid-contact method.

Results

It is easy to demonstrate that the physical structure of the seedball of many varieties of sugar beets delays germination. Whole seedballs germinate more slowly than notched seedballs, in which a small hole is made to expose the seedcoat of each true seed (Table 1 and Figure 2). Notching the seedball apparently accelerates water uptake and decreases the imbitional force required to pry loose the "seed cap" or lid covering the ovarian cavity of the seedball. Processing or removal of some of the corky substance of the seedball will also speed up germination.

Gemma (2) has attributed non-germination of *Beta patellaris* and *Beta procumbens* to the inability of the seed to push up the seed cap, even when it absorbs enough water to germinate.

[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, and the Michigan Agricultural Experiment Station. Approved for publication as Journal article No. 2066, Michigan Agricultural Experiment Station.

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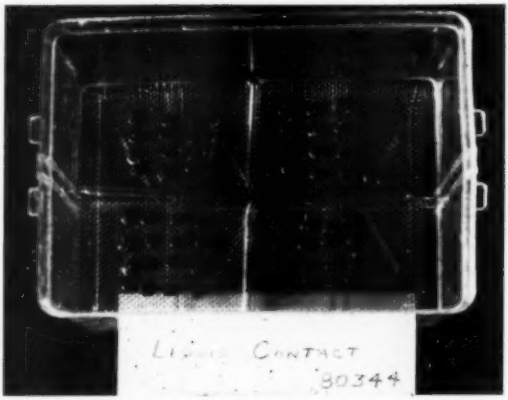

Figure 1.—View into plastic box showing general arrangement and size. Plastic box top removed. (Number of seedballs differs from experiments mentioned in text.)

Figure 2.—Effect of notching the seedball into each true seed on the speed of germination.

Table 1.—Effect of Notching Sugar Beet Seedballs on Speed of Germination by the Liquid-Contact Method.

	Accumulated Percentage Germination by Days[1]			
	1	1.5	2	5
Whole Seedballs	0.0	2.0	12.5	62.1
Notched Seedballs	31.6	65.4	76.5	—

[1] Based on 800 seedballs.

The role of seed-cap tightness in the physical hindrance of the seedball to germination has not been evaluated in commercial varieties. Some qualitative data on seed-cap tightness are available. These data were obtained from seedballs that had been in contact with water or nutrient solution for at least seven days. The caps over ovarian cavities containing ungerminated seeds were pried loose and placed into categories of tightness according to the estimated force required to loosen them. The results for five varieties of sugar beets are presented in Table 2. These qualitative data clearly indicate varietal differences in the tightness of seed caps and permit an approximate classification of

Table 2.—Tightness of Seed Caps Over Ungerminated Sugar Beet Seeds in Contact with Water or Nutrient Solution for at Least Seven Days.

Variety	Approximate Germination 5th Day	Seed Caps Examined	Tightness of Seed Caps Over Ungerminated Seeds		
			Tight	Intermediate	Loose
	%	No.	%	%	%
SP 5377-0 Monogerm	3	238	94.5	1.7	3.8
US 400, Acc. 1329	44	252	77.4	10.3	12.3
US 400, Acc. 1329	34	329	55.3	24.6	20.1
Acc. 32 (Kohls)	15	112	33.0	49.1	17.9
US 401	60	153	41.2	26.1	32.7
SP 5389-3	75	241	16.6	27.0	56.4

varieties on this basis. Data reported by the author (5) in a preliminary report on tightness of seed caps of variety US 400 were evaluated on a somewhat different basis and so are not strictly comparable, especially with respect to loose caps. The loose caps can be determined much more precisely than the tight or intermediate ones; therefore, emphasis should be directed toward this category. Relatively little is known about the influence of environmental factors on the tightness of seed caps. However, when cloned seed-bearing plants were grown at mean temperatures of 66° and 76° F. a greater proportion of the loose seed caps occurred on seedballs of plants grown at the higher temperature (7).

In variety US 400, the seed caps of a seedball may differ in relative tightness. Of 528 seedballs examined, 60 percent had all seed caps of the seedballs in a specific tightness category. The remaining 40 percent were in the mixed-tightness category, and nearly half of this mixed category contained both tight and loose seed caps on the same seedball.

Seeds of sugar beet have germinated more rapidly after periods of soaking followed by thorough drying prior to planting (3).

It is not known whether the improved germination results from (a) removal of inhibitory substances from the seedball, or (b) from loosening of the seed caps by the stresses induced by alternate wetting and drying. Soaking had no significant effect on loosening the seed caps covering ungerminated seeds of variety US 400. Soaking the seedballs of varieties US 400 and US 401 prior to germination by the liquid-contact method under osmotic stress did not alter the percentage of germination in any consistent manner in comparison with unsoaked seedballs. Coe[1] has observed that soaking of seedballs of different varieties prior to germination may be beneficial, may have no effect, or may even be detrimental to germination of seeds. It appears that soaking may be beneficial only when seedballs contain an abundance of inhibitory substances that must be diluted to a concentration that permits germination.

Three substances that may affect germination adversely have been isolated from the seedballs of sugar beets (1, 4, 8); however, only ammonia (8) and oxalic acid (4) have been identified as to their chemical nature. A qualitative test has indicated the presence of oxalic acid in the seedballs of variety US 400. Acidulation and calcium added to counteract the presence of ammonia and oxalic acid, respectively, failed to improve germination of US 400 in the liquid-contact method using nutrient solution of 10.1-atmosphere osmotic pressure.

In studies employing bulk seedlots of a given variety, whole seedballs almost without exception germinated more slowly than "naked seeds" (the true seeds freed of all maternal tissues exterior to the seedcoats) obtained from the same seedlot. When seed of variety US 401, harvested by individual plants, was compared for speed of germination, whole seedballs from a few plants actually germinated more rapidly than the "naked seeds" from seedballs of the same plants. Following classification of seedlots from 182 individual plants of US 401 for speed of germination, six of the most rapidly germinating lots were used in more detailed studies. The seeds were germinated by the liquid-contact method. The effect of the seedball on germination and growth is shown in Table 3. In these six progenies the seedball failed to retard germination, and in two progenies (50232, 50246) it actually hastened germination. Furthermore, the presence of the seedball stimulated the early growth of the seedlings.

Although seedballs of progeny number 50098 did not hasten germination markedly, it was the only progeny of these six rapid germinators with an adequate supply of seed available for further experimentation. Because of fungal contamination, experiments in which naked seeds were placed in the same

[1] Unpublished data.

Table 3.—Effect of the Seedball on Germination and Growth of Selections of Sugar Beet Variety US 401 by the Liquid-Contact Method.

Progeny Number	Percentage Germination by Hours				Fresh Weight of 50 Seedlings in 6 Days	
	24	48	72	96	Gms.	Ratio (Whole/Naked)
50086:						
Whole seedballs	14	88	89	89	1.92	2.04
Naked seeds	36	77	89	94	0.94	
50098:						
Whole seedballs	26	91	96	96	1.70	1.98
Naked seeds	25	54	69	76	0.86	
50141:						
Whole seedballs	27	92	95	96	2.60	1.82
Naked seeds	49	94	98	98	1.43	
50195:						
Whole seedballs	12	78	82	87	1.95	1.65
Naked seeds	32	74	85	89	1.18	
50232:						
Whole seedballs	83	96	98	100	1.75	1.73
Naked seeds	46	77	88	92	1.01	
50246:						
Whole seedballs	62	89	93	96	2.12	2.26
Naked seeds	21	76	78	87	0.94	

Data for each progeny are based on 100 seedballs or naked seeds except for the whole seedballs of 50232 for which only 80 were used.

plastic box with either whole seedballs or the broken portions of seedballs have been unsatisfactory. In the liquid-contact method of germination, seedlings no longer in direct contact with the seedballs developed as rapidly as those still in contact with the seedballs. On the basis of this observation, the effect of a water extract of the seedballs on germination was investigated. The extract was prepared as follows: 1.75 grams of air-dried broken portions of seedballs (maternal tissues exterior to seed coats) of progeny 50098 were soaked in 20 milliliters of distilled water for 18 hours in a refrigerator. This mixture was then kept for two hours at room temperature with occasional gentle shaking. After 20 hours of contact, approximately 15 milliliters of the extract was decanted. This extract was then placed in the freezing compartment of a refrigerator and partially frozen. About six milliliters of this concentrated extract was poured off. Five milliliters of extract was added to 200 milliliters of the nutrient solution of 10.1-atmosphere osmotic pressure. If one assumes that the extract contained no solutes, by dilution, it would lower the osmotic pressure of the nutrient solution

less than half an atmosphere. This reduction in osmotic pressure could not account for the marked increase in fresh weight observed (Table 4). The presence of the extract increased the fresh weight of the seedlings 21.7 percent, but failed to speed up germination significantly. Further experimentation is planned.

Table 4.—Effect of an Extract from Seedballs of a Selected Sugar Beet Plant of Variety US 401 on Germination and Seedling Growth by the Liquid-Contact Method.

Treatment	Percentage Germination[1] by Hours			Fresh Weight of 50 Seedlings in 6 Days
	24	48	72	
				Grams
Whole seedballs in nutrient solution	13	92	95	1.77
Naked seeds in nutrient solution	43	86	94	1.06
Naked seeds in nutrient solution plus extract from seedballs	63	93	97	1.29

[1] 100 seedballs or naked seeds per treatment.

Discussion

If the endosperm has developed normally and the embryo is viable, then the speed of germination of the sugar-beet seed is controlled largely by physical and chemical factors in the maternal tissues (seedball) surrounding the true seeds (6). Although the physical structure of the seedball generally retards germination, presumably by impeding water uptake, some exceptions have been observed (Table 3). The tightness of seed caps and thickness of the inner layers of the maternal tissues surrounding the seed vary sufficiently among varieties to suggest heritable differences.

Where the seed itself does not possess any form of dormancy, as in the commercial sugar beet varieties, the speed of germination may be related to the rate at which water is imbibed by the seed. The tighter the seed cap the greater the delay in germination. However, because of the presence of inhibitory substances in the seedball, it is extremely difficult to evaluate separately the contribution of the physical and chemical factors to the delay in germination. Although little experimental evidence is currently available, environmental factors operating during maturation of the seedball also apparently influence the physical characters of the seedball which affect the speed of germination. The data in Table 2 suggest that even after five days in favorable conditions for germination the tightness of the seed cap may affect the percentage of germination, especially in the monogerm variety SP 5377-0.

Inhibitory substances in the seedball probably play the major role in the retarded germination observed in the sugar beet (1, 3, 4, 6, 8). Results from the present study indicate, perhaps for the first time, that the seedball of certain progenies may be nearly free of inhibitory substances. In some of these same progenies, the data (Tables 3 and 4) also indicate the presence of some substance which appears to stimulate germination and early seedling growth. In the investigation of an inhibitory substance isolated from a water extract of the seedballs of sugar beets, DeKock, et al. (1) reported that cress seeds were inhibited by a water extract of this substance. If, however, the seeds so treated were washed and set out for germination, they reported that the rate at which the seeds germinated was remarkably rapid and the hypocotyls were much stouter than those of the unwashed controls. From this observation they conclude that some phase of the growth process is being retarded whereas other phases may proceed normally or be stimulated. They did not indicate whether one or more substances may be involved. All evidence from the present investigation indicates that the inhibitory substance or substances differ from the stimulatory substance.

Since the nutrient solution employed in the present study contains a copious supply of calcium and is moderately acidic even after a period of contact with the seedballs, the soluble oxalic acid would be precipitated as calcium oxalate and the ammonia would be tied up as an ammonium salt. Thus, these two inhibitory substances probably have little effect on germination of seedballs in contact with this nutrient solution.

Speed of germination seems to be a heritable trait (6). The pattern of performance in variety US 401 suggests that a genetic mechanism is controlling the amount of these chemical substances in the seedball. At present, no information is available on the modifying influence of environmental factors on these chemical substances.

Through the technique of progeny selection, it is hoped that genetically stable progenies, that will permit identification of the chemical factors involved, can be obtained. Perhaps specific chemical tests could then be utilized to locate the desirable attributes in various progenies. By proper selection, we hope to discard the detrimental seed characters which delay germination.

Summary

Experiments were designed to evaluate some of the effects of the physical and chemical factors of the seedball of the sugar beet on the speed of germination. The liquid-contact method of germination was used.

1. The maternal tissues of the seedball usually hinder germination, since seedballs notched to expose a portion of each true seed germinate more rapidly than whole seedballs of the same variety.

2. Varieties differ in the degree of tightness of the seed cap over the true seed.

3. Soaking of sugar beet seeds in water prior to germination may reduce the percentage of germination, particularly when the seeds are germinated by the liquid-contact method under osmotic stress.

4. Seedballs from some plants of sugar beet variety US 401 apparently contain so little chemical inhibitors that germination is not delayed.

5. Whole seedballs from two plants of US 401 germinated more rapidly than naked seeds obtained from the same seedlots, indicating a stimulating effect of the seedball. The growth of the seedlings also was stimulated by the seedballs.

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The Use of Systemics Di-Syston and Thimet to Control Virus Yellows in California

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Received for publication September 15, 1958

The virus yellows disease of sugar beets, formerly found only in Western Europe, is fast becoming a major problem in certain sugar beet growing areas of the United States. Since the initial report by Coons (2)[2] in 1952 that the disease was evident in the United States, it has developed into a serious disease problem. Evidence compiled from damage appraisal studies (1, 3, 4) has shown that the disease causes considerable losses in yields and sucrose percentages in sections of Colorado, and that it is especially serious in parts of California. During the 1956 growing season the disease was observed in the South San Joaquin Valley of California, damage being most prevalent to sugar beets grown in Kern County. The following season (1957) damage was even more severe and widespread within the county. A need for some type of control of the disease was evident.

Since resistant varieties are not available and the causal virus is persistent in green peach aphids, control or partial control of the disease involves the control of this vector. Thus the following test was designed to test the effectiveness of systematic insecticides in controlling the green peach aphid (*Myzus persicae*) and, at the same time, to measure losses caused by the disease.

Materials and Methods

Thimet 44-D [44% O, O-diethyl S-(ethylthiomethyl) phosphorodithioate] and Di-syston [50% O, O-diethyl S2-(ethythio) ethyl phosphorodithioate] were the two systemics used both as seed treatments and sidedress applications. These two materials can be absorbed by the root system and then be translocated to the stems and leaves. Once in the leaves they may be picked up by the aphid at feeding time. The test conducted near Wasco, California, (Kern County), was planted February 25, 1957, using the sugar beet variety US 22. Plots consisted of twelve 32-inch rows 100 feet long. Fifty feet of the four center rows of each plot were harvested and used for yield data. Two samples of about 35 pounds per plot were analyzed at harvest for sucrose and purity.

The test was designed to include the following treatments and an untreated check in a 5 x 5 Latin Square.

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[2] Numbers in parentheses refer to literature cited.

1. Check—no treatment.
2. Thimet 44-D—seed treatment (4 lbs. of 44% Thimet material per 100 lbs. of seed).
3. Thimet 44-D—seed treatment (4 lbs. of 44% Thimet material per 100 lbs. of seed) plus two sidedressing applications 8 and 14 weeks after planting with 25 lbs. of 4% granular Thimet per acre per application.
4. Di-syston—seed treatment (4 lbs. of 50% Di-syston per 100 lbs. of seed).
5. Di-syston—seed treatment (4 lbs. of 50% Di-syston per 100 lbs. of seed) plus two sidedressing applications 8 and 14 weeks after planting with 60 and 32.8 lbs. of 2.5% granular Di-syston per acre per application, respectively.

Results

On March 27, approximately one month after planting, an inspection of the test plot indicated the possibility of a slight stunting of the beet seedlings in the seed treated plots. A small amount of tip burn was evident on the cotyledons of seedlings from the treated seed compared to the cotyledons of seedlings from untreated seed. Two months after planting, the stunting of the plants and the tip burn on the cotyledons was not evident.

Green peach aphids were not visible until April 28, or two months after planting, but no more than two aphids per plant were found then. The first sidedress application was applied at this time. The plots requiring a second sidedressing received the application on June 13, three and one-half months after planting. At this time no aphids were visible in any of the plots. One week later the plots were inspected and again aphids were not found in any of the plots. The absence of aphids prevailed through harvest.

Virus yellows symptoms were not found in any of the plots until June 13, three and one-half months after planting. At this time yellowing had become apparent but not too prevalent. Some individual beets showed symptoms of virus yellows in both the treated and untreated plots. One week later yellowing had begun to appear in a general manner in the entire experiment, and no differences among the treatments were noted.

At the final inspection of the plots on July 7, two months prior to harvest, a yellows count was attempted, but infection appeared to be 100 percent, as judged by symptoms on the older leaves. New or young leaves appeared green at this time. This situation prevailed until the plots were harvested on September 4, 1957.

Yield data: Table 1 presents the yield in tons of roots per

Table 1.—Effect of Systemic Insecticide Treatments on the Percent Sucrose, Percent Thin Juice Purity, Tons Per Acre, and Gross Sugar Per Acre at Wasco, California in 1957.

Treatment	Treatment Means			
	% Sucrose	% Thin Juice Purity	Tons/Acre	Gross Sugar/Acre
1. Check	12.1	87.2	11.8	2853
2. Thimet (seed trt.)	12.3	85.8	12.7	3099
3. Thimet (seed trt. plus sidedress)	12.2	88.1	14.2	3452
4. Di-syston (seed trt.)	12.1	89.0	14.3	3444
5. Di-syston (seed trt. plus sidedress)	11.8	89.1	14.6	3439
General Mean	12.1	87.8	13.5	3257
$SE\bar{x}$	.255	.739	.879	—
L.S.D.	NS	2.3	NS	NS

acre, gross sugar per acre, percent sucrose, and percent thin juice purity. Results shown in Table 1 indicate that significant differences were obtained for percent thin juice purity only. Treatment 2 (Thimet 44D seed treatment) was lower in purity than treatments 3, 4, and 5, but did not indicate a significant decrease in purity compared to the check. The difference in purity between treatments 2 and 3 is unexplainable.

Gross sugar per acre and tons per acre for all treatments did not differ significantly from the check. However, the test using systemic insecticides to determine tonnage and sugar losses due to virus yellows indicated a trend toward higher yields.

Discussion

It may be noted that the plots were sidedressed 8 and 14 weeks after planting. While the 8 week sidedress application may have been beneficial to the control of the green peach aphid, some aphids were actually present on the plants at the time of this first sidedress application. Evidence presented by McLean in 1957 (5) indicated that the systemic insecticide Thimet used on table beet seed plants was not effective in leaves 21 days after application as a seed treatment. Thus, the sidedress treatments in this test may possibly have been applied too late to have been available to the green peach aphid at all times after planting. The results obtained, however, seem promising enough to warrant further study of the use of systemic insecticides to control the green peach aphid on sugar beets.

Summary

1. The use of two systemic insecticides (Di-syston and Thimet 44-D) as seed treatment and seed treatment plus two sidedress applications was studied to determine actual losses in tonnage and sugar caused by the virus yellows disease of sugar beets in Kern County, California, for the 1957 growing season.

2. Visual inspection indicated both insecticides caused some tip burning on the cotyledons from treated seed. Damage from sidedressing the sugar beets with the insecticides was not evident.

3. Green peach aphids were found in the plots two months after planting. One and one-half months later no aphids were found. The absence of aphids prevailed through harvest.

4. Further inspection revealed individual beets had virus yellows symptoms four months after planting. One week later all the plots indicated a general yellowing effect. Infection appeared to be 100 percent two months before harvest.

5. Data compiled from the test indicated a trend toward increases in the mean tons per acre and mean gross sugar per acre with the use of the systemic insecticides. However, these increases were not statistically significant. A significant difference among treatments for thin juice purity percentage was obtained at the 5% level, but was not entirely explainable.

6. Data derived from the test point to negative results with these two systemic insecticides to control virus yellows of sugar beets, but the possibility of too long a time lapse between systemic insecticide treatment applications and a trend of increased yield with more applications seem to warrant further experimentation.

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Some Chemical and Physiological Characteristics of Inbred Lines of Sugar Beets

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Received for publication August 28, 1958

Previous to 1952, sugar beet lines possessing both high sucrose percentage and curly-top resistance had not been fully developed. Selection from a broad self-fertile genetic base at Salt Lake City in 1953 and 1954, followed by progeny tests in 1955 and 1956, resulted in the development of some high-sugar, curly-top-resistant lines. These self-fertile lines were utilized for a study of chemical and physiological characters, with emphasis on nitrogen determinations of petioles and sodium content of roots in relation to sucrose percentage and other quantitative measurements. A statistical study of some of these quantitative measurements was made in the hope of finding better methods of identifying superior genotypes, and extensive correlation coefficients were calculated to determine possible relationships between the different characters.

Materials and Methods

Well drained, highly productive Taylorsville loam (2)[2] soils were chosen for selection and evaluation work in 1954, 1955 and 1956. The fields were located at Granger, Utah. A long-time crop rotation plus regular applications of manure and phosphate fertilizer, maintained a yielding capacity of sugar beets approaching and often exceeding 30 tons per acre on the experimental sites. In spite of this highly productive soil, petiole samples taken at an early harvest date in 1954 showed low nitrate-nitrogen content. One small-scale test employing a late nitrogen fertilizer application was made in 1954 (see Table 2). In 1955 and 1956 liberal applications of nitrogen fertilizer were made over a chosen portion of the replicated plots of each field during the growing season.

The test in 1954 was devoted to a collection of inbred lines of sugar beets for preliminary selection work. Some lines were grown only in single plots while others appeared in a second replication. An early harvest, September 21, was made of approximately twenty individual beets from each of the inbred lines. Fifteen measurements were made or calculated on each beet in each line. Petiole samples from five recently matured leaves per beet (9) were taken at harvest. For the small-scale

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[2] Numbers in parentheses refer to literature cited.

test with the heavy late nitrogen application, variety 342.400, representing the broad, self-fertile genetic base, was utilized, and individual beets were harvested and petiole samples taken November 2.

In 1955 five inbred lines were selected for further study of differences from the standpoint of sucrose percentage, amino N and sodium content. These varieties were planted in randomized blocks with 40 ft. plots at two levels of fertility, normal and high, with three replications at each level. The high-fertility level was obtained by adding two applications of ammonium nitrate at the approximate rate of 120 pounds of nitrogen per acre July 20 and again on August 27. A composite sample of ten roots was taken from each plot October 3 and another sample on November 4. Five additional individual beets, selected for uniformity of size and shape, were taken from each plot November 4. Petiole samples were taken from each selected plant for nitrate-nitrogen analysis.

In the 1956 test there were two nitrogen levels with six varieties replicated three times in 4-row plots. The high-fertility level received two supplemental applications of ammonium nitrate; one at the rate of 75 pounds nitrogen per acre on June 20, the other at the rate of 90 pounds nitrogen per acre on July 26. Two hundred pounds per acre of 24-20-0 fertilizer were applied to all plots before planting. Twenty consecutive beets were taken from the row with the best competitive stand in each 4-row plot. The early harvest date, September 24, was chosen in order to obtain a higher nitrate-nitrogen content in the petioles.

The lines tested in 1955 and 1956 resulted from the intensive selection work done in 1954, except for the commercial variety US 41 used as a check in 1956. The list of varieties was as follows:

US 41	Intermediate sugar-type commercial variety
Line 163	Low-sugar type
Line 163A	Higher in sugar than line 163
SLC 117	A monogerm inbred, intermediate-sugar type
CT9	Yield type with proven combining ability (3)
CT5	Intermediate-sugar type
CT5 subline	Similar to CT5 but more uniform
CT8	High-sugar type
CT7	Low-sodium, high-sugar type

Petiole nitrate-nitrogen analyses were made on dried samples at the University of California, Berkeley, California, by methods previously described (9). Other chemical analyses were made at the US Department of Agriculture field laboratory in Salt

Lake City, Utah, by methods also described (7). Amino N in the beet, as glutamine, was determined on clear filtrate (26 grams pulp digested in 177 ml. H_2O and 0.7 grams Hornes dry lead), by the Stanek-Pavlas method using the spectrophotometer as the absorption instrument. Na and K in the beet were determined on part of same filtrate by use of the flame spectrophotometer. Respiration measurement is reported in milligrams (mg.) of CO_2 per kilogram (kg) (fresh weight) per hour using a fresh 10-gram cylindrical beet root section. Total nitrogen in each dried petiole sample was determined by the Kjeldahl method. Percent purity as reported is the ratio of sucrose to total soluble solids, often referred to as apparent purity. Percentage of total soluble solids was determined with a dipping-type refractometer. Percent sucrose was obtained by direct polarization of clear filtrate.

All information was punched on IBM cards (5). The cards were punched locally and sent to the Computing Laboratory, Biometrics Services, Agricultural Research Center, Beltsville, Maryland, where means, standard deviations, and correlation coefficients were calculated by machine processing. Variance analyses and coefficients of variability were completed by hand calculation.

Experimental Results

Means of Quantitative Measurements

Yearly means of all measurements for 1954, 1955, and 1956 are shown in Table 1. The data in Table 1 for the year 1954 are those obtained at the early harvest date, September 21.

Table 1.—Yearly Means, All Measurements, with Varieties and Fertility Levels Combined.

Code	Measurement	Year		
		1954	1955	1956
1.	Beet weight in grams	1150	1480	1310
2.	Percent sucrose	14.6	17.1	14.2
3.	Amino N in beets, p.p.m.	3600	3700	7600
4.	Na in beets, p.p.m.	275	270	230
5.	Respiration rate CO_2/kg/hr (mg)	112		
6.	K in beets, p.p.m.	3060	2630	3110
7.	Sugar content, grams (beet wt. × percent sucrose)	165	252	182
8.	Percent solids		19.2	16.6
9.	Nitrate-nitrogen in petiole, p.p.m.	520	865	4084
10.	Percent purity		89.1	85.0
10A.	Top weight, grams (1954)	743		
11.	Number of leaves (based on weight of 5 leaves)	43		
12.	Ratio of petiole to top weight	51.7		
12A.	Total N in petiole, p.p.m.		13.800	
13.	Length of petiole, cm.	22.7		
14.	Space code (rated 1 to 9)	2.2		
	Number of observations (individual beets)	530	150	720

on normal-fertility plots. The nitrate-nitrogen in the petioles was only 520 p.p.m. which is considered rather low for an early harvest date.

Table 2.—Effect of Heavy Late Application of Nitrogen on Sugar Beet Analyses, 1954.

Fertility Level	Number Beets	Root Analyses						Petiole Analyses
		Beet Weight	Sucrose	Amino N	Na	K	Purity	NO_3-N
		Lbs.	Percent	PPM	PPM	PPM	Percent	PPM
Heavy Nitrogen[1]	20	3.15	14.98	5600	427	3318	84.6	4539
Normal	20	3.21	17.72	2300	197	2369	89.8	185

[1] Nitrogen applied August 27, 300 lbs. per acre of ammonium sulphate, and September 10, 300 lbs. per acre of ammonium nitrate, harvested November 4, 1954.

Table 2 shows spectacular effects from a late, heavy application of nitrogen in 1954 with the beets harvested November 2. The sucrose dropped from 17.72 percent on the normal-fertility level to 14.98 percent on the high-fertility level, with a corresponding drop from 89.8 to 84.6 in percent purity. The late, heavy application of nitrogen resulted in high increases of amino N, Na, and K in the beet roots and a tremendous increase in nitrate-nitrogen in the petioles, from 185 p.p.m. on normal fertility to 4539 p.p.m. on the high-fertility level. Beets grown at normal fertility also decreased in petiole nitrate from 520 p.p.m. on September 21 to 185 p.p.m. on November 2.

Table 3 gives the means of varieties for 1955 and 1956. In both years line CT7 proved to be different from other lines because of its low Na (Figure 1) and K content in the root, a surprisingly low nitrate-nitrogen content in the petioles, but high amino nitrogen in the roots (Figure 2) and high total nitrogen in the petioles.

The 1955 data shown in Table 4 are from individual beets. Data from ten-beet composite samples taken from the same plots in 1955 are shown in Table 5. Here the striking effect of harvest date and fertility level are easily seen. The lowest sucrose was obtained at the early harvest, October 3, with high nitrogen fertilization. The highest sucrose was obtained at the late harvest, November 4, with normal fertility. The striking behavior of CT7 with low Na but high amino N content is again illustrated.

Table 3.—Variety Means.

Variety	No. Beets	Root Analyses								Petiole Analyses	
		Beet Weight	Sucrose	Amino N	Na	K	Sugar Content	Solids	Purity	NO_3-N	Total N
		Grams	Percent	PPM	PPM	PPM	Grams	Percent	Percent	PPM	PPM
1955 Test											
Line 163A	30	1650	17.0	2600	410	3020	278	18.9	89.5	1130	12300
CT9	30	1420	16.7	3600	310	2690	238	18.7	89.3	740	13300
SLC 117 mm	30	1670	17.1	3100	290	2480	282	19.1	89.7	870	12600
CT8	30	1490	18.0	3000	220	2530	266	20.0	89.8	1070	14700
CT7	30	1150	16.9	6200	110	2450	194	19.3	87.2	520	16000
MEAN, all varieties	150	1480	17.1	3700	270	2630	252	19.2	89.1	870	13800
LSD 5% point		250	0.72	1220	81	243	38	0.72	1.33	NS	1330
1956 Test											
US 41	120	1390	13.1	7700	480	3800	181	15.7	83.1	6075	
Line 163	120	1580	12.0	10700	200	3700	189	14.7	81.4	4949	
CT5	120	1280	14.7	6400	250	2860	186	17.0	86.2	4185	
CT5 subline	120	1250	14.4	6100	150	2970	179	16.6	86.7	4170	
CT8	120	1270	15.8	5700	200	2810	197	18.1	87.2	3218	
CT7	120	1080	15.1	9100	103	2520	161	17.6	85.7	1934	
MEAN, all varieties	720	1310	14.2	7600	230	3110	182	16.6	85.0	4084	
LSD 5% point		210	0.80	1660	68	255	NS	0.80	1.91	1227	

Table 4.—Effect of Fertility Level, 1955.

Variety	Fertility	No. Beets	Root Analyses								Petiole Analyses	
			Beet Weight	Sucrose	Amino N	Na	K	Sugar Content	Solids	Purity	NO_3-N	Total N
			Grams	Percent	PPM	PPM	PPM	Grams	Percent	Percent	PPM	PPM
			Mean for variety at each fertility level									
Line 163A	Normal	15	1630	17.9	2200	280	2800	291	19.9	90.3	660	11400
	High	15	1670	16.0	3000	540	3230	265	18.0	88.8	1610	13100
CT9	Normal	15	1500	17.3	2900	280	2710	257	19.2	90.1	400	12300
	High	15	1350	16.2	4200	340	2680	218	18.3	88.5	1070	14300
SLC 117 mm	Normal	15	1820	17.6	2500	230	2380	313	19.3	90.8	490	11500
	High	15	1520	16.6	3700	340	2570	251	18.8	88.6	1240	13700
CT8	Normal	15	1440	18.4	2600	160	2370	263	20.3	90.5	390	13300
	High	15	1540	17.6	3300	280	2690	268	19.7	89.1	1750	16100
CT7	Normal	15	1200	17.2	5200	100	2270	206	19.5	88.4	350	14800
	High	15	1110	16.5	7100	120	2630	183	19.2	86.1	690	17100
			Mean for all varieties at each fertility level									
	Normal	75	1520	17.7	3100	210	2500	266	19.6	90.0	460	12700
	High	75	1440	16.6	4300	320	2760	237	18.8	88.2	1270	14900
	Difference		—80	—0.9[a]	1200[a]	90[a]	260[a]	—29[a]	—0.8[a]	—1.8[a]	810[a]	2200[a]

[a] Significant at 1% level

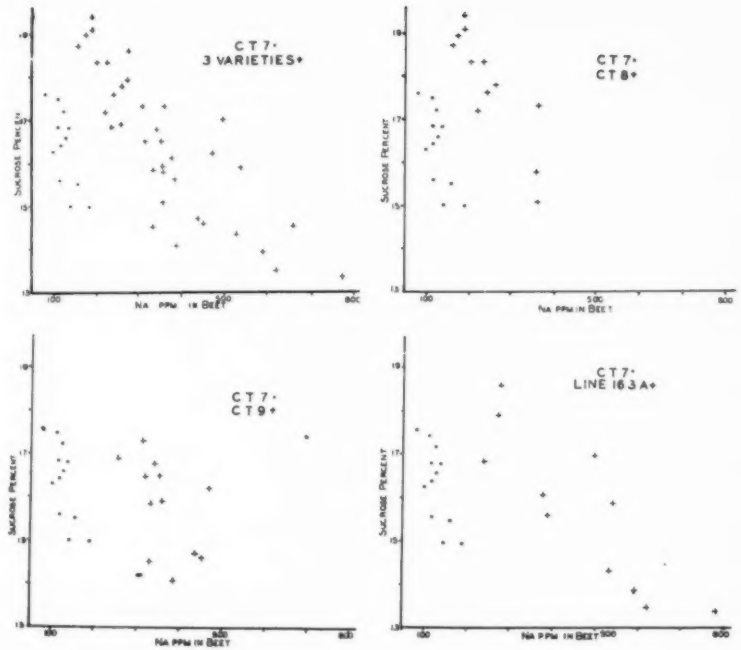

Figure 1.—Scatter diagrams comparing inbred CT7 (.) and three other inbred lines (+) in relation to percent sucrose and Na in the beet. Data are from 10-beet composite plot samples taken in 1955, with two dates of harvest, September 21 and November 2, on two fertility levels with three replication plots at each level, making a total of twelve plots and twelve 10-beet composite samples. See Table 5 for tabulation of data.

The same 1955 data for sucrose percentage and Na content shown in Table 5 are illustrated by scatter diagrams in Figure 1. In these scatter diagrams the positions for CT7 are so different from those of the other varieties that there is no overlapping. CT7 occupies a unique position in all comparisons.

Table 4 shows means for fertility levels and varieties for 1955. The mean nitrate-nitrogen in the petioles was 460 p.p.m. at the normal fertility level, and 1270 p.p.m. at the high fertility level. The difference between fertility levels was not so great in 1956 (Table 6); the normal fertility averaged 3622 p.p.m. and the high fertility 4545 p.p.m. The higher nitrate-nitrogen in petioles in 1956 was due to the earlier date of harvest, September 24, as compared with the late date of harvest, November 4, in 1955.

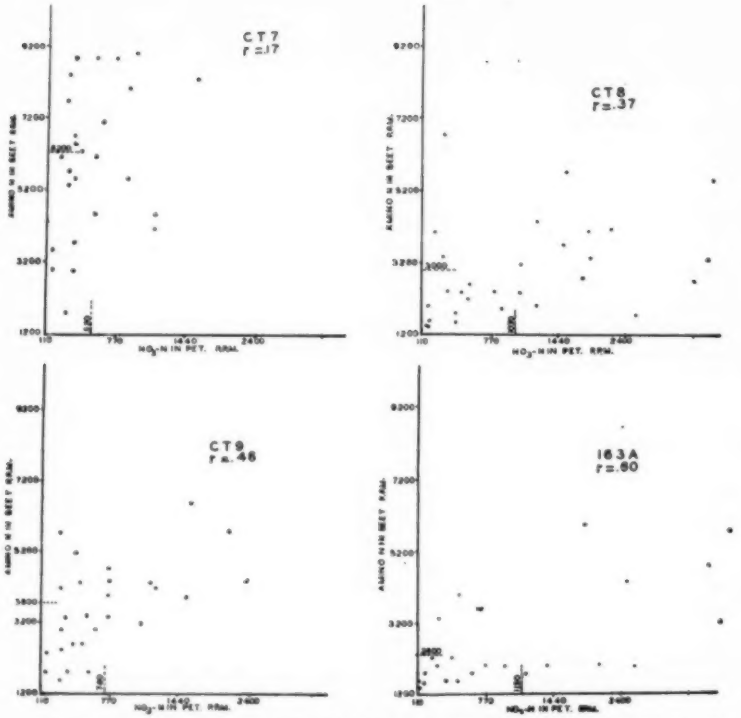

Figure 2.—Scatter diagrams showing relationships of Amino N in the beet root to Nitrate N in the petiole for four varieties: CT7, CT8, CT9, and line 163A; representing 1956 data from individual beets taken from two fertility levels, three plots at each level and five beets from each plot, a total of 30 beets of each variety.

Statistical Interpretation of Means

The measurements from individual beets shown in Tables 3, 4, and 6, were subjected to variance analysis and the statistical interpretation is presented in Table 7 for 1955 and 1956 data. The variance ratios, or calculated F values in terms of errors variance, show highly significant differences due to varieties. In interpreting these results one should bear in mind that the varieties were selected principally for differences in sucrose content. Sodium content was also considered to some extent. The varieties were not selected for the other chemical constituents; yet, for the most part, highly significant differences were obtained between varieties for all chemical constituents.

In 1956 (Table 7), there were highly significant differences due to varieties for all measurements, including petiole nitrate

analyses. In 1955 the results were much the same but with a less pronounced effect of variety on petiole nitrate-nitrogen. The decided decreases in sucrose at the high-fertility levels (Tables 4 and 6) are shown to be highly significant by the variance ratios (Table 7). The influence of fertility level on most other chemical analyses was also highly significant.

Table 5.—Means from 10-beet Composite Samples in 1955 with Three Replicated Plots at Each Fertility Level.

Variety	Weight	Sucrose	Purity	Amino N	Na	K
	Lbs.	Percent	Percent	PPM	PPM	PPM
			Oct. 3 harvest—High N			
Line 163A	29.2	13.6	87.5	3400	660	3850
CT9	28.9	14.6	87.6	4600	410	3400
CT8	20.1	16.0	88.9	4100	310	3240
CT7	21.5	15.2	84.9	7400	150	2970
Avg.		14.8	87.2	4875	384	3365
			Oct. 3 harvest—Normal fertility			
Line 163A	29.2	15.3	90.3	2100	430	3500
CT9	23.3	15.1	88.6	2500	380	3120
CT8	18.0	18.1	92.8	1600	230	2610
CT7	19.3	16.4	90.0	5000	130	2850
Avg.		16.2	90.4	2800	292	3020
			Nov. 4 harvest—High N			
Line 163A	21.8	15.7	82.8	5300	480	3710
CT9	22.8	16.2	85.9	4400	380	2790
CT8	20.5	18.1	87.0	3500	250	2540
CT7	18.8	16.6	83.9	7800	120	2560
Avg.		16.6	84.9	5250	307	2900
			Nov. 4 harvest—Normal fertility			
Line 163A	25.7	17.8	87.3	2500	350	2860
CT9	24.5	17.0	87.5	3100	300	2850
CT8	20.7	18.9	87.4	1900	190	2300
CT7	21.3	17.3	84.8	5800	100	2300
Avg.		17.8	86.8	3325	235	2578

In general the differences due to fertility level were larger in 1955 than in 1956. This is accounted for by the fact that the fertility gradients were more distinct in 1955. Unfortunately in 1956 there were differences in degree of fertility among the different plots on the normal fertility level. Fertility level had a distinct effect on the content of nitrate-nitrogen in the petioles in 1956, but the F value of 8.52, due to fertility level, was lower than the F value of 13.50, due to variety. Significant differences in beet weight due to fertility level were not obtained in either year. This was due to the fact that the nitrate-nitrogen was above the critical level (1000 p.p.m.) and so would not affect growth differentially (10). However, significant reductions in sucrose percentage do occur at nitrate concentrations higher than the critical level affecting beet weight.

Table 6.—Effect of Fertility Level, 1956.

Variety	Fertility	No. Beets	Root Analyses								Petiole Analyses
			Beet Weight	Sucrose	Amino N	Na	K	Sugar Content	Solids	Purity	NO_3-N
			Grams	Percent	PPM	PPM	PPM	Grams	Percent	Percent	PPM
			Mean for variety at each fertility level								
US 41	Normal	60	1380	13.1	6800	560	3620	181	15.9	82.5	5887
	High	60	1400	13.0	8600	410	3980	182	15.5	83.7	6277
Line 163	Normal	60	1590	12.4	11600	210	3590	196	15.5	80.1	5022
	High	60	1570	11.6	9800	180	3810	182	14.0	82.8	4863
CT5	Normal	60	1200	14.9	5500	260	2680	177	17.3	85.9	3463
	High	60	1350	14.4	7200	240	3040	194	16.7	86.5	4921
CT5 subline	Normal	60	1130	14.8	5100	140	2780	166	17.0	86.8	3463
	High	60	1380	14.0	7000	170	3160	193	16.2	86.6	4877
CT8	Normal	60	1240	16.3	5100	210	2640	200	18.8	86.6	2323
	High	60	1290	15.3	6200	190	2990	194	17.4	87.8	4113
CT7	Normal	60	1090	15.4	8900	110	2440	167	18.2	84.3	1616
	High	60	1060	14.7	9300	100	2600	155	17.0	87.0	2251
			Mean for all varieties at each fertility level								
Average	Normal	360	1270	14.5	7200	250	2960	181	17.1	84.4	3622
	High	360	1340	13.8	8000	220	3260	183	16.1	85.7	4545
	Difference		70	—0.7[2]	800	—30	300[2]	2	—1.0[2]	—0.7[1]	923[2]

[1] Significant at 5% level
[2] Significant at 1% level

[2] Significant at 1% level

Table 7.—F Values from Variance Analyses.

Variable	Degrees of Freedom	Root Analyses								Petiole Analyses	
		Beet Weight	Sucrose	Amino N	Na	K	Sugar content	Solids	Purity	NO_3-N	Total N
Calculated F values, 1955											
Fertility	1	1.06	25.96[2]	10.41[2]	21.66[1]	11.85[2]	6.60[1]	15.14[2]	19.19[2]	26.63[2]	28.69[2]
Varieties	4	6.09[2]	4.12[2]	12.08[2]	16.73[2]	8.15[2]	8.32[2]	4.23[1]	5.37[2]	2.01	12.02[2]
Varieties × Fertility	4	.91	1.05	.33	2.72	1.15	0.99	1.53	0.21	1.14	0.27
Calculated F values, 1956											
Fertility	1	1.37	8.04[2]	3.10	2.94	18.31[2]	0.11	21.03[2]	6.51[1]	8.52[2]	
Varieties	5	5.48[2]	25.96[2]	12.70[2]	32.78[2]	35.48[2]	2.42	22.03[2]	12.15[2]	13.50[2]	
Varieties × Fertility	5	5.54[2]	0.27	1.52	1.63	0.28	1.12	0.71	0.72	0.94	

[1] Significant at 5% level
[2] Significant at 1% level

The F value (Table 7) measuring the interaction between variety and fertility level, with the exception of beet weight in 1956, shows no evidence of significance. No significant interaction with sucrose percentage is indicated. However, Table 4 indicates that the sucrose percentage of the higher sugar lines CT8 and CT7 may have been less affected by fertility level than the other varieties in 1955. Here (Table 4) the reductions in sucrose percentages at the high versus the normal fertility levels were: For CT8, 0.8; for CT7, 0.7; for CT9, 1.1; for SLC 117, 1.0; for 163A, 1.9. These figures indicate a tendency for an interaction between fertility level and variety, but the differences were not great enough for statistical significance.

Coefficients of Variability

Table 8 shows that the greatest variability was in measurements of weight, amino N, Na, sugar content and nitrate-nitrogen in the petiole. Variability was decidedly lower for percent sucrose, percent solids, and purity. The coefficients of variability at each fertility level were roughly the same for most measurements except for nitrate-nitrogen in the petioles. The variability of petiole nitrate was greater on normal fertility because the nitrogen was near the deficiency level. Some individual plants were deficient while others were not.

Correlations with Petiole Nitrate

Table 9 shows r values obtained in 1955 by variety and in 1956 by variety and fertility level correlating nitrate-nitrogen in the petioles with eight different root measurements. In 1955 petiole nitrate correlations with root analyses for amino N, Na, and K varied considerably according to variety. Line CT7 with high amino N and low Na and K, which in previous tables was shown to be different from other lines in actual values, also appears to have given somewhat different correlation values. The three correlations with petiole nitrate, 0.17 for amino N (Figure 2), 0.28 for Na, and 0.06 for K, are low positive values but not statistically significant. The corresponding correlations with some of the other varieties are highly significant in the positive direction. In 1956 (Table 9) the results with CT7, correlating nitrate-nitrogen in the petiole with amino N in the root, show a statistically significant negative r value, whereas a positive r value was expected based on results with other varieties.

Correlations with Sodium

Correlations between Na content and other quantitative measurements are shown in Table 10 for 1955 and 1956. Na is correlated negatively with percent sucrose (Figure 1), solids,

Table 8.—Coefficients of Variability[1].

Variety	No. Beets	Root Analyses								Petiole Analyses	
		Beet Weight	Sucrose	Amino N	Na	K	Sugar Content	Solids	Purity	NO_3-N	Total N
					1955						
Line 163A	30	26.6	8.4	56.1	59.0	15.8	26.0	8.0	2.0	40.5	19.2
CT9	30	21.0	6.0	39.2	23.5	11.0	20.9	5.0	1.8	94.6	12.8
SLC 117 mm	30	31.7	7.0	31.9	36.6	17.6	30.0	6.5	2.1	94.8	19.3
CT8	30	25.5	5.0	44.3	39.1	15.4	22.9	4.4	1.7	79.9	14.0
CT7	30	18.7	5.6	36.3	26.4	16.4	18.5	4.4	2.5	66.9	15.3
Normal	75	29.8	5.5	21.1	46.5	16.2	27.9	4.9	1.8	105.6	17.0
High	75	27.7	6.7	32.4	60.6	16.7	26.6	8.2	1.8	70.3	17.6
All plots combined	150	22.0	6.8	33.8	61.0	17.1	27.8	6.1	2.25	94.7	18.9
					1956						
US 41	120	37.2	13.5	41.4	63.5	14.7	38.6	10.5	4.8	19.5	
Line 163	120	33.6	13.5	40.9	45.5	13.9	35.3	11.6	5.7	9.0	
CT5	120	30.4	7.5	31.1	38.4	18.2	29.8	6.6	3.4	21.3	
CT5 subline	120	31.9	9.1	30.6	40.0	16.3	31.2	7.8	4.3	28.7	
CT8	120	35.6	8.6	43.3	39.0	22.7	33.1	7.5	3.8	28.5	
CT7	120	29.6	5.6	26.5	33.0	13.7	28.4	6.1	2.8	73.5	
Normal	360	36.9	13.1	54.7	88.4	22.7	33.9	10.2	4.8	64.5	
High	360	34.3	12.8	32.7	64.6	21.3	33.3	10.5	4.8	47.1	
All plots combined	720	35.5	13.1	44.3	81.2	22.5	33.5	10.8	4.9	56.1	

[1] Based on the standard deviation for numbers of beets shown.

Table 9.—Correlations, By Variety, Obtained Between Nitrate-Nitrogen In Petiole and Other Quantitative Characters.

Variety	Fertility Level	No. Beets	Nitrate-Nitrogen in Petiole Correlated with: Beet Weight	Percent Sucrose	Amino N	Na	K	Sugar Content	Percent Solids	Purity	Total N in Petiole
			1955 r values								
Line 163A	Combined	30	—.26	—.32	.60[2]	.37[1]	.16	—.35[1]	—.21	—.50[2]	.72[2]
CT9	Combined	30	—.02	—.23	.46[2]	.04	—.21	—.09	—.16	—.31	.44[1]
SLC 117 mm	Combined	30	—.06	—.32	.09	.44[1]	.10	—.13	—.26	—.27	.73[2]
CT8	Combined	30	—.02	—.39[1]	.37[1]	.63[1]	.37[1]	—.11	—.20	—.65[2]	.76[2]
CT7	Combined	30	—.19	—.40[1]	.17	.28	.06	—.30	—.35[1]	—.30	.45[1]
			1956 r values								
US 41	Normal	60	—.22	—.78[2]	.28[1]	.54[2]	.29[1]	—.47[2]	—.75[2]	—.67[2]	
	High	60	—.06	—.18	.11	.11	—.03	—.11	—.23	—.04	
Line 163	Normal	60	.23	—.23	.07	.29	.35[1]	.18	—.08	—.29[1]	
	High	60	.07	.10	.04	.09	.27[1]	.10	.14	0	
CT5	Normal	60	—.01	—.33[1]	.16	.28	.36[1]	—.07	—.34	—.17	
	High	60	—.18	.09	.17	—.16	.05	—.15	.21	—.17	
CT5 subline	Normal	60	.36[2]	—.70[2]	.54[2]	.32[1]	.69[2]	.20	—.71[2]	—.32[1]	
	High	60	—.02	—.20	.09	.54	.15	—.06	—.21	—.03	
CT8	Normal	60	.09	—.35[1]	.34[1]	.03	.36[1]	.22	—.30[1]	—.29[1]	
	High	60	.20	—.45[2]	.25[1]	.17	.25[1]	.10	—.42[2]	—.21	
CT7	Normal	60	—.06	—.48[2]	—.25[1]	.42[2]	.26[1]	—.16	—.53[2]	.02	
	High	60	—.10	—.44[2]	—.01	.47[2]	.26[1]	—.16	—.52[2]	—.04	

[1] Significant at 5% level
[2] Significant at 1% level

and purity. Most of these strong negative correlations are very highly significant. Na is correlated positively with K content and in general with amino N, also with nitrate-nitrogen in the petioles and with total nitrogen in the petioles.

Discussion

The most striking evidence from the three years' work reported is that each inbred line of sugar beets may have its own peculiar chemical and physiological characteristics. These peculiar characteristics are shown by the correlation relationships. Two measurements correlated in one way for one inbred line may be correlated in quite another way with another inbred (8) (Figures 1 and 2).

The strong negative correlation between Na content and sucrose percentage reported by Doxtator (1) was well confirmed. In connection with this correlation it was interesting to note that Na content was strongly affected by both variety and nitrogen fertility level. In 1954 a late heavy application of nitrogen (Table 2) more than doubled the Na content in the beet roots. The effect of variety was equally striking. Variety CT7 (Tables 3, 4, 5, and 6) was extremely low in Na in the root. Nearly four times as much Na was taken up by certain other varieties. Early in the work, before the relationship between Na and sugar percentage was as well understood as it is now, some consideration was given to Na as a direct means of selection. It is likely that selection of low Na individuals in a heterogeneous beet population would result at the same time in higher sucrose percentage types. At first one gained the impression that all the higher sugar lines were low Na lines. However, after three years of experience it became clear that no fast rules could be followed. CT8 consistently produced a higher sugar percentage than CT7 but the Na content for CT8 was nearly twice as great as that for CT7 (Tables 3, 4, 5, and 6). It would, therefore, be very unwise to depend upon low Na as a true index of high-sucrose genotypes.

To a certain extent the picture is the same for some of the other chemical analyses. Nitrate-nitrogen in the petioles, although very strongly associated with the nitrogen level of the soil, was also influenced by variety. Under similar environments less than half as much nitrate-nitrogen was found in the petioles of CT7 as in some of the other varieties. The nitrate content in the petioles of the high-sugar line CT8 was nearly twice as high as that for CT7 (Tables 3, 4, and 6).

The exceptional characteristics of CT7 may lead to interesting assumptions regarding underlying physiological relationships. The low nitrate-nitrogen but high total nitrogen in the

Table 10.—Correlations by Variety Obtained Between Na in Beet and Other Quantitative Characters.

Variety	Fertility Level	No. Beets	Na in the Beet Correlated With: Beet Weight	Percent Sucrose	Amino N	K	Sugar Content	Percent Solids	Percent Purity	NO_3-N Petiole	Total N in Petiole
			1955 *r* values								
Line 163A	Combined	30	.10	—.78[2]	—.05	.54[2]	—.15	—.80[2]	—.10	.37[1]	.10
CT9	Combined	30	.24	—.64[2]	.08	.29	.06	—.66[2]	—.27	.04	.18
SLC 117 mm	Combined	30	.33	—.69[2]	.41[1]	.62[2]	.21	—.58[2]	—.51[2]	.44[1]	.48[2]
CT8	Combined	30	.02	—.46[2]	—.18	.54[2]	—.08	—.40[1]	—.31	.63[2]	.54[2]
CT7	Combined	30	.04	—.25	—.12	.06	—.03	—.31	—.02	.28	.24
			1956 *r* values								
US 41	Normal	60	.10	—.76[2]	—.09	.25[1]	—.21	—.18[2]	—.59[2]	.54[2]	
	High	60	.04	—.55[2]	.19	.21	—.11	—.54[2]	—.39[2]	.11	
Line 163	Normal	60	.00	—.57[2]	.10	.27[1]	—.16	—.33[1]	—.58[2]	.29	
	High	60	—.01	—.74[2]	.20	.36[2]	—.32[1]	—.72[2]	—.57[2]	.09	
CT5	Normal	60	.14	—.66[2]	.26[1]	.45[2]	—.01	—.63[2]	—.49[2]	.28	
	High	60	.08	—.68[2]	.12	.39[2]	—.11	—.62[2]	—.17	—.16	
CT5 subline	Normal	60	.17	—.62[2]	.32[1]	.54[2]	.01	—.55[2]	—.49[2]	.32[1]	
	High	60	.13	—.60[2]	.13	.60[2]	—.04	—.40[2]	—.38[2]	.54	
CT8	Normal	60	—.06	—.42[2]	.18	.32[1]	—.15	—.40[2]	—.32[1]	.03	
	High	60	—.04	—.17	.00	.06	—.06	—.20	.00	.17	
CT7	Normal	60	.18	—.44[2]	—.12	.17	.10	—.41[2]	—.17	.42[2]	
	High	60	.16	—.47[2]	—.08	.26[1]	.10	—.41[2]	—.12	.47[2]	

[1] Significant at 5% level
[2] Significant at 1% level

petioles of CT7, and high amino nitrogen in the roots indicate that this line may be able to metabolize nitrate more rapidly than the other varieties. These special characteristics of CT7 may be interpreted as a precaution also against too much generalization concerning correlation values, without considering each variety separately. When one line behaves differently from other lines in the association of chemical and physiological characteristics correction values may be a distinguishing feature of the line.

The variance ratios expressed as F values in Table 7 give strong evidence that the varieties investigated reacted more or less the same at both normal and high fertility. Coefficients of variability (Table 8) were also relatively independent of fertility level except for petiole nitrate.

The *r* values were not large for petiole nitrate-nitrogen correlated with amino N, Na, K, and purity; however, some of these relatively low *r* values were statistically significant. Some of the low values could be due to curvilinear relationships instead of straight-line relationships (10). A curvilinear relationship may be more applicable for petiole nitrate correlations with beet weight, K in beet and sugar content, etc., where few significant *r* values were obtained. Stronger *r* values might be obtained if multiple or partial correlations were calculated.

The expense and time required for progeny tests make the breeder very conscious of doing everything he can to make the greatest gains possible in his initial selection work. Powers (4) has illustrated this point and has developed statistical methods which help separate genetic from environmental variability. With more information on the association between chemical and physiological characteristics, it may be possible that correlation values could be arrived at which may help the breeder.

Petiole nitrate measurements would undoubtedly be more valuable if one could make analyses at different specific times during the growing season. The final date when the beet is harvested and selected is perhaps the least desirable date for taking the petiole samples. Petiole nitrate taken some time before harvest may give a much better index of available nitrogen to each individual beet (10).

A comparison of harvest dates in 1955 illustrates a physiological problem quite independent of variety. Table 5 shows that the sugars from the late harvest were higher than from the early harvest while the purities were lower. This is contrary to what one may expect. This unexpected decrease in purity is not accounted for by an increase in Na and K but may be partly accounted for by an increase in Amino N.

The present information shows that there are distinct differences between different lines in sodium, potassium, and amino nitrogen, as well as the other usually measured constitutents. Percentage raffinose (11) has been shown to be a heritable character. High respiration rate of beet root tissues is another undesirable character because of the greater storage loss from such beets (6). Unpublished studies have shown that respiration rate is also a heritable character. Little information is available at present on the individual effects of some of the other constituents on processing quality, although impurities in processing liquors have increased in recent years. More information is needed regarding the relative importance of these impurities. It seems quite certain that plant breeding can result in considerable alteration in these factors. However, an overall economic evaluation must be made because breeding for a reduction in some impurities may reduce the acre yield of roots.

Summary

Inbred lines of sugar beets were shown to have their own peculiar chemical and physiological characteristics. In general there was a high negative correlation between sucrose percentage and Na content of the root but there were striking genotypic differences. Inbred CT7 from root analyses was relatively high in sucrose, extremely low in Na, and high in amino N. In petiole analyses CT7 was low in nitrate-nitrogen but high in total N. Inbred CT8 was higher in sucrose than CT7 but contained nearly twice as much Na in the root and nearly twice as much nitrate-nitrogen in the petiole. On the basis of the nitrogen relationships some physiological explanations are attempted to account for the genotypic differences.

Measurements of nitrate-nitrogen in the petioles, amino N in the roots, and Na content in the roots were greatly influenced by high nitrogen fertilization, but for these same measurements varietal differences were just as striking. In Na content, a four- or five-fold difference between low Na types and high Na types was not uncommon. In general sucrose percentage was less variable than some of the other measurements and was influenced more by nitrogen fertilization than by variety, although there were also very significant varietal differences.

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Distribution of Cyst- and Gall-Forming Nematodes of Sugar Beets in the United States[1]

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Received for publication June 1, 1958

Introduction

Sugar beets in the United States have grown intermittently, then continuously, for more than 120 years. Today, plant parasitic nematodes stand as one of the major problems confronting the beet-sugar industry. Since the first report of the sugar-beet nematode, *Heterodera schachtii* Schmidt, 1871, in a sugar beet field near Lehi, Utah, about 1895, known areas of infestation have increased in size and number. (2, 8, 15, 23)[4].

Species of root-knot nematodes, *Meloidogyne* spp. Goeldi, 1887, are a great cause of trouble in California and are frequently encountered in other sugar-beet-growing areas.

Until recently Cobb's root-knot nematode, *Nacobbus batatiformis* Thorne and Schuster, 1956, had been mistaken for root-knot nematode, *Meloidogyne* spp., in sugar-beet-producing areas on the eastern slope of the Rocky Mountains (25).

Recent studies (1, 3) have afforded the opportunity of determining the incidence and distribution of these forms. The cooperation of interested workers and their generous sharing of information have enabled this distribution report to be as current and accurate as possible.[2]

Procedure

The Beet Sugar Development Foundation conducted two studies on the incidence of plant parasitic nematodes which encompassed 86 percent of the sugar-beet-producing area.[3] Samples of sugar beets and contiguous soils were examined for nematodes from 14 sugar-beet-producing states as follows: California, Colorado, Idaho, Iowa, Kansas, Michigan, Minnesota, Montana, Nebraska, North Dakota, Oregon, South Dakota, Washington, and Wyoming.

Distribution and allocation of samples were based on sugar-beet acreage. Sampling was timed to collect in the selected areas at approximately the middle of the growing season. The collection of samples was delegated to sugar company fieldmen who were familiar with their particular territories. In one study conducted in California, Colorado, Kansas, and Michigan, fields

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[2] Those who deserve special thanks for their cooperation are A. L. Taylor, Gerald Thorne, Harold J. Jensen, A. Morgan Golden, D. J. Raski, E. C. Jorgensen, M. L. Schuster, G. Steiner, Harold W. Reynolds, and the Agriculturists and Fieldmen of the beet-sugar industry.

[3] The assistance and cooperation of the officials of Agricultural Research Service of the United States Department of Agriculture and the officials and personnel of the beet-sugar industry which helped make these studies possible are gratefully acknowledged.

with a known or suspected nematode infestation were selected and four samples were taken from each field. Sample A was taken from the apparent center of an infested area as determined by sugar-beet growth. Sample B was taken midway from the apparent center to the apparent edge of the infested area. Sample C was collected at the apparent edge of the infested area. The collection of Sample D was from an apparently unaffected area of the field.

In the other study, fields selected for sampling could have a known history of nematode infestation, a suspected nematode infestation or no nematode problems. Two samples were collected from each field. Sample A was taken from the center of an infested area or the center of the field if there was no evidence of a nematode infestation. Sample B was taken from a location in the field at least 50 yards distant from Sample A.

Fields selected for sampling were of a representative soil type used for growing sugar beets in the fieldman's particular area. Fields were avoided where nematode control practices by soil fumigation had been employed. If a field contained more than one infested area, only one such infestation was sampled.

The method of sampling was as follows: each sample consisted of one whole sugar beet, top included, and one quart of soil. Sugar-beet roots were dug rather than pulled to preserve the maximum number of secondary roots. Soil surrounding the beet one foot square and to the depth of the actively growing

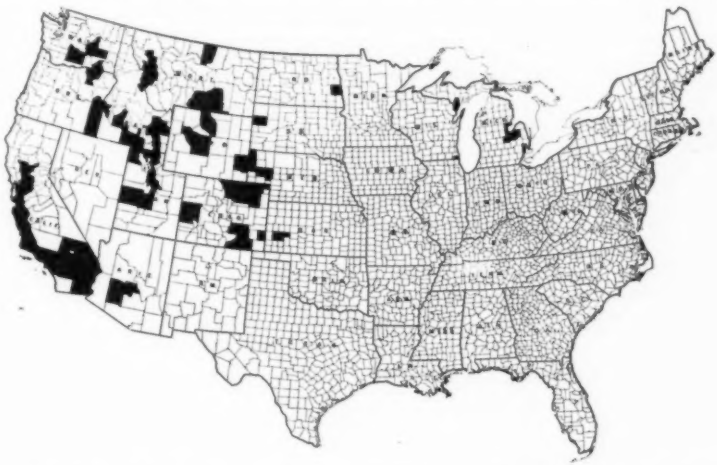

Figure 1.—Distribution of the sugar-beet nematode, *Heterodera schachtii* by counties, as observed on sugar beets in the United States.

Figure 2.—Distribution of the cotton root-knot nematode, *Meloidogyne incognita acrita*, by counties, as observed on sugar beets in the United States.

fibrous roots or ten inches, whichever was greater, was thoroughly mixed on a clean surface for each sample. A one-quart aliquot of soil was then taken.

The nematodes were separated from the soil using the gravity-screening technique and preserved in 5-dram vials in five percent formaldehyde. The Baermann funnel method was combined with the screening during the second study to obtain residue free samples. Spear-form nematodes were selected from the samples that had only been screened. A medicine dropper technique was employed for rapid slide preparation of the residue free samples (3).

During the first study, samples of beet were taken from the fibrous roots, the tap root, the crown, the petioles, and the leaf blade. The beet samples were then preserved and stained in steaming acid fuchin in lacto-phenol. Second study beet samples were collected only when nematode presence was observed or suspected.

Results and Discussion

The distribution of the nematodes is given by counties in each of the states where they occur. *H. schachtii* distribution is shown in Figure 1. The distribution of *M. incognita acrita* Chitwood, 1949, is shown in Figure 2. *M. hapla* Chitwood, 1949, distribution on sugar beets is shown in Figure 3. Reports of unidentified gall-forming nematodes is shown in Figure 4. Dis-

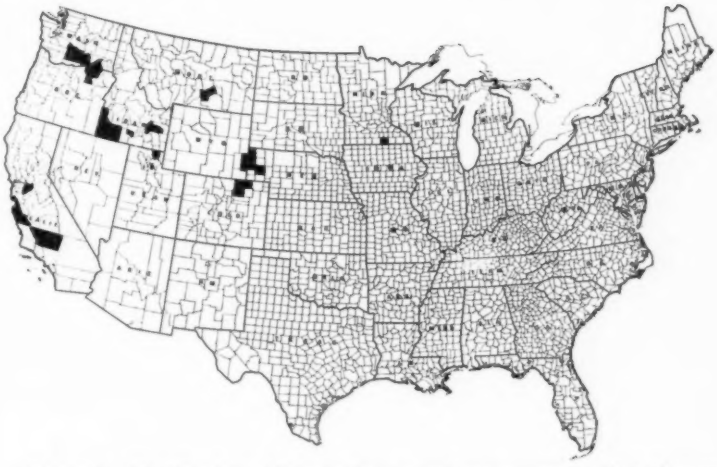

Figure 3.—Distribution of the northern root-knot nematode, *Meloidogyne hapla*, by counties, as observed on sugar beets in the United States.

Figure 4.—Distribution of the root-knot nematode, *Meloidogyne*, spp., by counties, as reported on sugar beets in the United States. See text for explanation of other possible identification.

tribution of *N. batatiformis* as found on sugar beets is shown in Figure 5.

Reports of *Meloidogyne* species in eastern Colorado and Kansas can probably be ascribed to *N. batatiformis* which forms

Figure 5.—Distribution of Cobb's root-knot nematode, *Nacobbus batatiformis*, by counties, as observed on sugar beets in the United States.

similar galls on sugar-beet roots. An intensive study of all gall-forming nematodes on sugar beets in Colorado has revealed only one *M. hapla* infestation near Milliken in Weld county. This field had a mixed population of *M. hapla* and *N. batatiformis* infesting sugar beets. Mixed populations of *M. hapla*, *N. batatiformis*, and *H. schachtii* were observed on sugar beets in the western sugar-beet-producing counties of Nebraska.

Thorne (23) reported the known *H. schachtii* distribution in 1952. The present investigations have complemented and supplement his report. Information sources of nematode distribution not observed by the author are cited below.

Known infestations of *H. schachtii* are as follows (Figure 1):

Arizona—*H. schachtii* has been reported on sugar beets in the Salt River Valley of Maricopa county by Reynolds (15, 16).

California—The known distribution of *H. schachtii* extends to 21 counties in California: Alameda, Colusa, Contra Costa, Imperial, Los Angeles, Monterey, Orange, Riverside, Sacramento, San Benito, San Bernardino (13, 22), San Joaquin, San Luis Obispo, Santa Barbara, Santa Clara, Santa Cruz (9), Solano, Sutter, Ventura, Yolo, and Yuba (9).

Colorado—Sixteen counties in Colorado are known to have infested sugar-beet fields: Adams, Boulder, Crowley (10, 18, 22), Delta, Larimer, Las Animas, Logan, Mesa (10, 22), Montrose, Morgan, Otero, Prowers (27), Pueblo, Sedgwick, Washington (26), and Weld.

Idaho—Sugar-beet fields in 14 Idaho counties are known to be infested: Bannock, Bingham, Bonneville, Canyon, Cassia, Elmore, Franklin (10, 22), Gooding, Jefferson, Jerome, Madison, Minidoka, Payette, and Twin Falls.

Kansas—*H. schachtii* has been observed in Finney and Kearney (27) counties.

Michigan—Six counties in Michigan contain infested sugar-beet fields: Arenac (12), Bay, Macomb (23), Menominee (23), Saginaw, and Tuscola.

Montana—Distribution of known infestations extends to nine counties in Montana: Big Horn, Blaine, Carbon, Lake, Missoula, Ravalli, Stillwater, Treasure (11), and Yellowstone.

Nebraska—In Nebraska, Garden (6), Morrill (6, 10, 22), Scotts Bluff, and Sioux counties are known to have infested sugar-beet fields.

North Dakota—One lightly infested field has been observed in Cass county.

Oregon—*H. schachtii* infestations have been observed in Malheur and Umatilla counties.

South Dakota—Several moderately to heavily infested fields have been encountered in Butte county.

Utah—Nine counties in Utah are known to have infested sugar-beet fields (23): Box Elder, Cache, Davis, Millard, Salt Lake, Sanpete, Sevier, Utah, and Weber.

Virginia—The reported presence of *H. schachtii* on Polygonum in Virginia has subsequently been diagnosed as *H. weissii* Steiner, 1953 (19, 20).

Washington—In Washington, Adams, Grant, Walla Walla, and Yakima counties have *H. schachtii* infested sugar-beet fields.

Wisconsin—Kenosha county has been reported infested (24).

Wyoming—Big Horn, Fremont, Goshen, Platte (10, 22), and Washakie counties are known to have infested sugar-beet fields.

Root-knot nematode, *Meloidogyne* species, infestations on sugar beets are as follows (Figures 2, 3, 4):

California—*M. incognita acrita* has been observed on sugar beets in Butte (14), Imperial, Kern, Madera, Sacramento, San Joaquin, Santa Clara, Tulare, and Yolo counties.

M. hapla has been found on sugar beets in Kern, Monterey, San Joaquin, Santa Cruz and Yolo counties.

One infestation of *M. javanica* (Treub, 1885) Chitwood, 1949, has been observed in San Joaquin county.

Infestations of *Meloidogyne* species on sugar beets have been reported from Contra Costa (24), Fresno (9), Kings (9), Los Angeles (13, 24), Merced (9), Orange (13, 24), Riverside (13),

San Benito (9), San Bernardino (13), San Luis Obispo (13), Santa Barbara (13, 24), Solano (9), Stanislaus (9), Sutter (9), Ventura (13, 24), and Yuba (9) counties.

Colorado—One field of sugar beets lightly infested by *M. incognita acrita* has been observed in Montrose county. *M. hapla* has been observed with *N. batatiformis* on sugar beets in one field in Weld county. Reports of *Meloidogyne* species in Adams (4), Larimer (21), Logan (5), Morgan (26), Otero (27), Prowers (27), Pueblo (27), and Washington (26) counties are probably mistaken observations of *N. batatiformis* on sugar beets.

Idaho—*M. hapla* has been observed on sugar beets in Bingham, Canyon, Jerome, and Owyhee counties.

Kansas—Reports of *Meloidogyne* species on sugar beets in Finney county (27) can probably be discounted as examination of sugar-beet roots has revealed only infestations of *N. batatiformis*.

Minnesota—A severe infestation of *M. hapla* has been observed on sugar beets in Freeborn county.

Montana—*M. hapla* has been observed on sugar beets from Yellowstone county.

Nebraska—*M. hapla* has been found on sugar beets in Morrill, Scotts Bluff, and Sioux counties. A species of *Meloidogyne* on sugar beets has been reported from Garden county (6).

Oregon—*M. hapla* has been observed on sugar beets in Umatilla county. A species of *Meloidogyne* has been reported on sugar beets in Malheur county (7, 17).

Utah—*M. hapla* has been observed on sugar beets in Cache and Davis counties (10). *Melcidogyne* species have been reported from Box Elder, Salt Lake, Utah, and Weber counties (24).

Washington—*M. hapla* has been observed on sugar beets in Benton, Walla Walla, and Yakima counties.

Wyoming—*M. hapla* has been observed in several sugar-beet fields in Goshen county.

Distribution on Cobb's root-knot nematode, *N. batatiformis*, on sugar beets has thus far been restricted to areas east of the Rocky Mountains (25). *N. batatiformis* has been observed on sugar beets in 14 counties and six states as follows (Figure 5):

Colorado—Infested sugar beet fields have been observed in Adams, Larimer, Logan, Morgan, Otero, Pueblo, and Weld counties.

Kansas—*N. batatiformis* has been found on sugar beets in Finney county.

Montana—Two lightly infested sugar-beet fields have been observed in Custer county.

Nebraska—Numerous infested sugar-beet fields have been observed in Morrill, Scotts Bluff, and Sioux counties.

South Dakota—A single infested field has been found near Vale in Butte county (10).

Wyoming—*N. batatiformis* has been observed in sugar beet fields in Goshen county.

Conclusions

The rise of this formidable nematode problem is little more than half a century of intensive sugar-beet culture in the United States should cause industry and growers to reappraise their attitudes and general practices.

The return of tare dirt to sugar-beet fields should be restricted. Machinery loaned or used on several fields should be properly cleaned. Small infestations should be contained by planting with a nonhost crop, leaving the remainder of the field for sugar beets. A proper sequence of crops should be followed to maintain soil fertility and retard or limit the rate of nematode population build-up. Where practicable, soil fumigation should supplement good farming and crop rotation. An attempt should not be made to substitute it for them.

The forcing of fields into extended rotations because of nematode infestation reduces the total acreage available for sugar-beet crops in any one year. A moderate reduction in total sugar-beet tonnage could have serious consequences for a factory district. An intensive and sustained program for conservation of sugar-beet-growing acreage should be initiated.

Summary

The known distribution of *H. schachtii* on sugar beets covers fields in 96 counties in 15 states. *M. incognita acrita* extends to ten counties in California and Colorado. *M. javanica* has been found on sugar beets in one field in California. *M. hapla* occurred on sugar beets in 22 counties in ten states. The known distribution of *N. batatiformis* covers 14 counties in six states.

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Some Effects of Gibberellin on Stem Elongation and Flowering in Sugar Beets[1] [2]

F. W. Snyder and S. H. Wittwer[3]

Control of both vegetative and reproductive responses in many crops is often approached with two opposite objectives. In production for fresh market or processing, a vegetative plant is often desired; while for a seed crop, bolting and flowering must be induced. Varieties of many biennial crops, including sugar beets, are being developed that are increasingly resistant to bolting. However, the more non-bolting the variety, the more difficult is seed production; and for the plant breeder the time required for the completion of a life cycle is increased.

Gibberellin may greatly modify the flowering responses of biennials and under some environmental conditions may promote earlier flowering and seed production. The cold requirement for stem elongation and flowering in *Hyoscyamus niger* (2)[4] and certain varieties of carrot, stock, and foxglove (3) may be completely replaced. Many cold-requiring biennials are also obligate long-day plants; and, while gibberellin may partially, or even completely replace the temperature requirement for flowering, it is unable to substitute for the photoperiodic requirement (2, 3). Gaskill (1) reported that a non-bolting variety (NB1) of sugar beet failed to bolt when gibberellin was applied in the absence of cold. However, when the sugar beets received partial "photothermal induction" (continuous illumination and temperatures of 45-46° F. for 43 days) followed by four foliar sprays at two-week intervals of 1000 p.p.m. of gibberellin, bolting as well as flowering and seed production occurred. Under conditions of partial photothermal induction alone, very few plants flowered (1). Thus, the critical thresholds of temperature and photoperiod for flower induction in sugar beets may be altered to facilitate earlier seed maturity in variety improvement programs and in commercial seed production. Gibberellin should prove particularly useful under field conditions where rigorous control of environment is not feasible.

Experiments were designed to determine the effect of gibberellin on the temperature and photoperiodic requirements for flowering in sugar beets.

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[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, and the Michigan Agricultural Experiment Station. Approved for publication as Journal Article No. 2358, Michigan Agricultural Experiment Station.

[2] The gibberellin used in these studies was the potassium salt of gibberellic acid (gibberellin A_3) supplied by Merck and Co., Rahway, N. J.

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[4] Numbers in parentheses refer to literature cited.

Experimental

Preliminary Studies

Response of Non-Induced Plants Grown at High Temperatures and Short Days—Roots of clone 55 CLE-17[3] were harvested in mid-October 1956, stored at 50 to 60° F. for two weeks in a root cellar, and then planted in pots and placed in a greenhouse for three weeks prior to treatment. Six plants each were sprayed to run-off with solutions containing 0.1 percent Tween 20 and gibberellin concentrations of 500, 1,000, 2,500 or 5,000 p.p.m. Half (three) of the plants were resprayed with the same concentrations 15 days later. Greenhouse temperatures approximating 65° F. were maintained at night, and plants were exposed to the prevailing mid-winter photoperiod of 9 to 11 hours.

Ninety days after treatment, the heights of the plants receiving the higher concentrations (2,500 and 5,000 p.p.m.) of gibberellin ranged from 4 to 6 feet with less stem elongation at 500 or 1,000 p.p.m. All, however, remained vegetative, the stalks finally terminating in aerial rosettes of varying heights. The second application induced very little additional stem elongation as compared with plants sprayed only once. The higher concentrations also caused the petioles of lower leaves to broaden and split longitudinally near the base and bend downward in a type of epinasty. The terminal leaves likewise appeared abnormal for several weeks following treatment. Lateral or side branches were markedly suppressed by the higher dosages. In plants producing more than one sprout, none of which possessed apical dominance, the shoots elongated more slowly and were shorter than on plants having a single shoot manifesting apical dominance.

Responses of Varieties and Clones Within Varieties—Individual plants within a sugar beet variety often exhibit marked differences in bolting. Accordingly, it was desirable to know if uniform bolting would result following treatment with gibberellin. Known quantities of gibberellin were applied in water solution to the crowns of non-induced plants of the clones listed in Table 1. Variability in stem elongation was as great between clones within a single variety as between clones of different varieties. This suggests that uniformity in bolting and seed production may not be easily realized with gibberellin and that optimal amounts for accelerating flowering may vary greatly among varieties, and even among clones within a variety.

[3] Clone derived from a plant with lineage of American Crystal No. 2-601 x H 149, supplied by Professor H. L. Kohls, Department of Farm Crops, Michigan State University.

Table 1.—Comparative Stem Elongation of Sugar Beet Varieties, and Clones of Plants Within a Variety, Following Treatment With Gibberellin.

Variety	Clone	Gibberellin Applied	Plants Treated	Elongation After 4 Months
		(Micrograms)	(Number)	(Centimeters)
54B4	50593	100	2	3 and 12
54B4	50599	100	2	41 and 57
54B4	50599	1,000	2	75 and 83
US 400	50624	1,000	2	1 and 1
US 400	50614	1,000	1	6

Transport of Gibberellin Bolting Stimulus in Plants with Double Shoots—Vegetative cuttings of sugar beet often develop more than one shoot. Such cuttings are admirably suited to study translocation of the gibberellin stimulus from the tip of one shoot to that of another. An initial study with clones E429-11m and E429-21m of the variety 54B4-17, indicated that gibberellin applied to one shoot had no effect on the bolting of non-treated shoots on the same plant.

In a more extensive experiment, 13 well established plants of clone 55 CLE-17, with shoots clearly separated, were selected. One hundred micrograms of gibberellin were placed on the primary or tip shoot of some plants and on the secondary or side shoot of others on October 24 and at approximately two-week intervals thereafter until January 9. The plants were watered without wetting the treated shoots and grown at the prevailing (9- to 11-hour) photoperiod, and at a night temperature approximating 65° F. By January 9, the treated shoots had elongated 18 to 27 inches. Differences were even more pronounced by March 4 (Figure 1). In contrast, the non-treated shoots, irrespective of position on the plant, failed to elongate. These results suggest a very localized effect of gibberellin on sugar beets.

Photoperiod and Temperature Studies

Effects of Gibberellin on Annual and Bienial Sugar Beets Grown Under 9- and 18-Hour Photoperiods—Seeds of annual (SL 9460) and biennial (US 400) sugar beets were sown September 9, 1957. Eight wooden flats (4″ x 16″ x 24″) containing vermiculite as the growing medium were used for each of the sugar beet types, and each flat contained 10 seedlings. Complete nutrient solution was applied to the vermiculite as needed. A photoperiod of 9 hours was maintained until October 14. On this date 4 to 5 leaves had developed, and four flats of 10 plants each of both the annual and biennial sugar beets were placed under a 9-hour and under an 18-hour photoperiod. Within

each environment, the plants in two of the four flats containing annual sugar beets, and two of those containing the biennial type were sprayed for the first time (October 14) with a solution containing 1,000 p.p.m. of gibberellin and 0.1 percent Tween 20 as a wetting agent. The spray treatments were repeated on November 7 and December 24. Equal numbers of flats containing non-sprayed plants in the two environments constituted the controls. A minimum night temperature approximating 55° F. was maintained throughout the experiment.

Figure 1.—A biennial sugar beet plant with two shoots that developed from a vegetative cutting. Shoot on left treated with 100 micrograms of gibberellin at approximately two-week intervals. Treatments initiated October 24 and continued until January 9. Photographed March 4, 1958. Plant grown under prevailing photoperiod and a night temperature of 65° F. Note the localized effect of gibberellin in the bolting of the treated shoot (left) in contrast to the absence of stem elongation in the non-treated shoot (right).

The effects of both photoperiod and gibberellin on stem elongation and flowering of the annual and biennial sugar beets are presented in Table 2 and Figure 2. Absence of flowering

Table 2.—Effects of Gibberellin on Flowering and Stem Elongation of Annual and Biennial Sugar Beets Grown Under Short (9-Hour) and Long (18-Hour) Photoperiods, and at a Minimum Night Temperature of 55° F.

Photoperiod	Gibberellin[1]	Time to Flowering[2]	Plants That Produced Normal Flowers	Plant Heights	
				Range	Mean
(Hours)		(Days)	(Percent)	(Centimeters)	
		Annual Sugar Beet (SL 9460)			
9	—	No Flowering	0	No Stem Elongation	
	+	No Flowering	0	46 to 62	54
18	—	80 to 105	100	100 to 143	119
	+	77 to 92	100	103 to 154	124
		Biennial Sugar Beet (US 400)			
9	—	—	0	1 to 146	62
	+	—	5[3]	52 to 211	140
18	—	151 to 206	90	62 to 180	130
	+	78 to 175	100	130 to 227	188

[1] Spray applications (1,000 p.p.m.) on October 14, November 7, and December 24.
[2] Number of days after initial (October 14) application of gibberellin.
[3] Inflorescence small and appeared after 199 days.

in the annual-type sugar beet under short days suggests an obligate long-day requirement for flowering, which was not satisfied by repeated treatment with gibberellin, even though marked stem elongation occurred (Table 2). Flowering of the annual sugar beets under a long photoperiod was accelerated by about 12 days with gibberellin, and seedstalks were slightly taller than those not treated with gibberellin.

As with annual sugar beets, most of the biennial plants flowered readily under the long photoperiod, but only one of 20 gibberellin-treated plants flowered under the short photoperiod. Flowering was generally accelerated under long days when the plants were treated with gibberellin (Figure 2), although some gibberellin-treated plants actually flowered at a date later than the first plants which flowered in the absence of gibberellin (Table 2). The data suggest that both annual and biennial sugar beets are obligate long-day plants. Gibberellin generally promoted flowering only when the plants were subjected to long days, although extensive stem elongation, without flowering, occurred subsequent to treatment of plants subjected to short days.

Effects of Gibberellin on Biennial Sugar Beets Grown at Various Photoperiods and Temperatures—Seeds of US 400 were sown January 2, 1958, and the seedlings grown in flats of vermiculite as in the previously described experiment. All plants

were maintained under a 9-hour photoperiod and at a temperature above 65° F. until February 27 when the gibberellin, photoperiod, and temperature treatments were initiated (Table 3). The initial (February 27) and subsequent (March 13 and 27, and April 10) gibberellin treatments consisted of sprays of 1,000 p.p.m. (plus 0.1 percent Tween 20) directed onto the growing tips. As in the previous experiment, two flats of 10 plants each were employed for each treatment.

Figure 2.—Effects of gibberellin and photoperiod on stem elongation and flowering in US 400 biennial (top) and SL 9460 annual (bottom) sugar beets. Left to right: 9-hour photoperiod; 9-hour photoperiod + gibberellin; 18-hour photoperiod; and 18-hour photoperiod + gibberellin.

Table 3.—Effects of Gibberellin, Photoperiod, and Temperature on Flowering and Stem Elongation of US 400 Sugar Beets.

Night Temperature	Photoperiod	Gibberellin[1]	Time to Flowering[2]	Plants That Produced Normal Flowers	Plant Heights	
					Range	Mean
(°F.)	(Hours)		(Days)	(Percent)	(Centimeters)	
55	9	—	No Flowering	0	No Stem Elongation	
		+	No Flowering	0	21 to 77	54
	12	—	No Flowering	0	No Stem Elongation	
		+	No Flowering	0	33 to 115	67
	18	—	96 to 115	65	0 to 105	60
		+	90 to 125	80	28 to 155	95
65	9	—	No Flowering	0	No Stem Elongation	
		+	No Flowering	0	12 to 84	36
	18	—	No Flowering	0	No Stem Elongation	
		+	62 to 117	40	41 to 194	106

[1] Spray applications (1,000 p.p.m.) on February 27, March 13 and 27, and April 10.
[2] Number of days after initial (February 27) application of gibberellin.

The data in Table 3 confirm the results of the earlier experiments. Flowering occurred only on plants exposed to an 18-hour photoperiod. At 55° F. and an 18-hour photoperiod, flowering was slightly accelerated by gibberellin, and there was an increase in the percent of plants which flowered. When the long-day requirement was satisfied, gibberellin induced 40 percent of the plants to flower, even at the "non-inductive" night temperature of 65° F. Of the plants which flowered, earliest flowering occurred on those sprayed with gibberellin and grown at a minimum night temperature of 65° F. and an 18-hour photoperiod (Table 3). Normal seedballs were subsequently produced by plants in which flowering was accelerated with gibberellin. Stem elongation was induced in all plants treated with gibberellin, irrespective of temperature or photoperiod (Figure 3).

Summary and Conclusions

Flowering in sugar beets may be accelerated and even induced with gibberellin, if treatments (repeated spray applications of solutions of 1,000 p.p.m. to the growing tips) are accompanied by exposure of the plants to a long (18-hour) photoperiod. At an 18-hour photoperiod and a night temperature of 55° F., gibberellin promoted earlier flowering of both annual (SL 9460 and biennial (US 400) sugar beets. No normal flowering

occurred at a 9-hour photoperiod. Appreciable flowering was induced with gibberellin (40 percent of treated plants) in US 400, a biennial variety of intermediate bolting tendency, when grown at a non-inductive temperature (65° F.) and simultaneously exposed to a long (18-hour) photoperiod. Considerable stem elongation in sugar beets resulted from all gibberellin treatments, irrespective of photoperiod and temperature. In plants produced from vegetative cuttings that had two or more terminals, the gibberellin stimulus for stem elongation was not transmitted from the treated shoot to one that was not treated.

Figure 3.—Effects of gibberellin, night temperature, and photoperiod on stem elongation and flowering in US 400 biennial sugar beets. A—65° F. minus gibberellin; B—65° F. plus gibberellin; C—55° F. minus gibberellin; D—55° F. plus gibberellin. Within each group (A, B, C, D), the plants on the left were exposed to a 9-hour photoperiod those on the right to an 18-hour photoperiod.

Marked variations occurred in the flowering behavior of types and varieties of sugar beets and in plants within the same variety exposed to the same environment. While flowering was generally accelerated and at times induced by spray applications of gibberellin, the variable flowering responses were not eliminated. Nevertheless, the results of these findings should prove useful for promoting earlier flowering and seed production as a means of accelerating variety improvement programs. Further testing under field conditions is needed before the value of gibberellin can be assessed in commercial sugar beet seed production.

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